VOLUMETRIC ANALYSIS
INCLUDING THE ANALYSIS OF GASES
WITH A CHAPTER ON SIMPLE
GRAVIMETRIC DETERMINATIONS

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PREFACE TO THE FIFTH EDITION

THE present work, the first edition of which was published in the year 1915, was written primarily in the interests of the author's own pupils. The object in view was to provide the student with a practical book, having a sufficient background of theoretical principles, which would occupy a position intermediate between that of the elementary works of necessarily limited scope and that of standard treatises on volumetric analysis.

The subsequent history of the book has amply demonstrated the soundness of the original plan. Various alterations and additions have been made in subsequent editions, more particularly in the fourth edition which was published ten years ago. A more drastic revision has been necessitated for the present edition, having regard to the rapid progress which has characterized every branch of analytical chemistry in recent years. Accordingly the author has embraced the opportunity of dealing with certain modern developments in volumetric analysis by adding a special chapter to the book. Among the various subjects which have been treated in this chapter, mention may be made of adsorption indicators, of oxidation and reduction indicators, of reduction with liquid amalgams, and of ceric sulphate as a quantitative oxidizing agent. These subjects should now constitute an essential part of the training of the student in volumetric methods.

Relatively few alterations have been made in the older part of the book, but two additions, one dealing with lead and the other with phosphates, have been added to the chapter on gravimetric determinations. A short chapter on the rudiments of gas analysis has also been added to the book.

In preparing this new edition, the author desires to acknowledge the information which he has derived from the study of larger works, particularly Kolthoff's *Volumetric Analysis*, Sutton's *Volumetric Analysis* revised by A. D. Mitchell, and *Neuere massanalytische Methoden* by E. Brennecke, K. Fajans, N. H. Furman, and R. Lang.
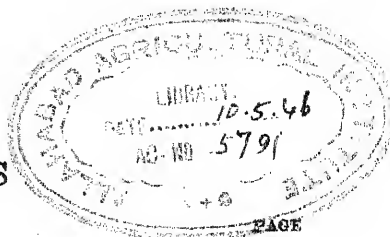
He also desires to thank several friends who have kindly helped him in various ways, particularly Dr Palmer, Fellow of St John's College, for criticizing the chapter on modern developments in volumetric analysis, and Dr McCombie, Fellow of King's College, for help with the chapter on the analysis of gases.
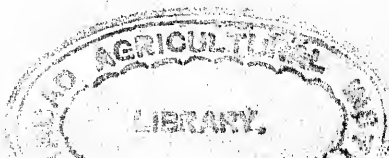
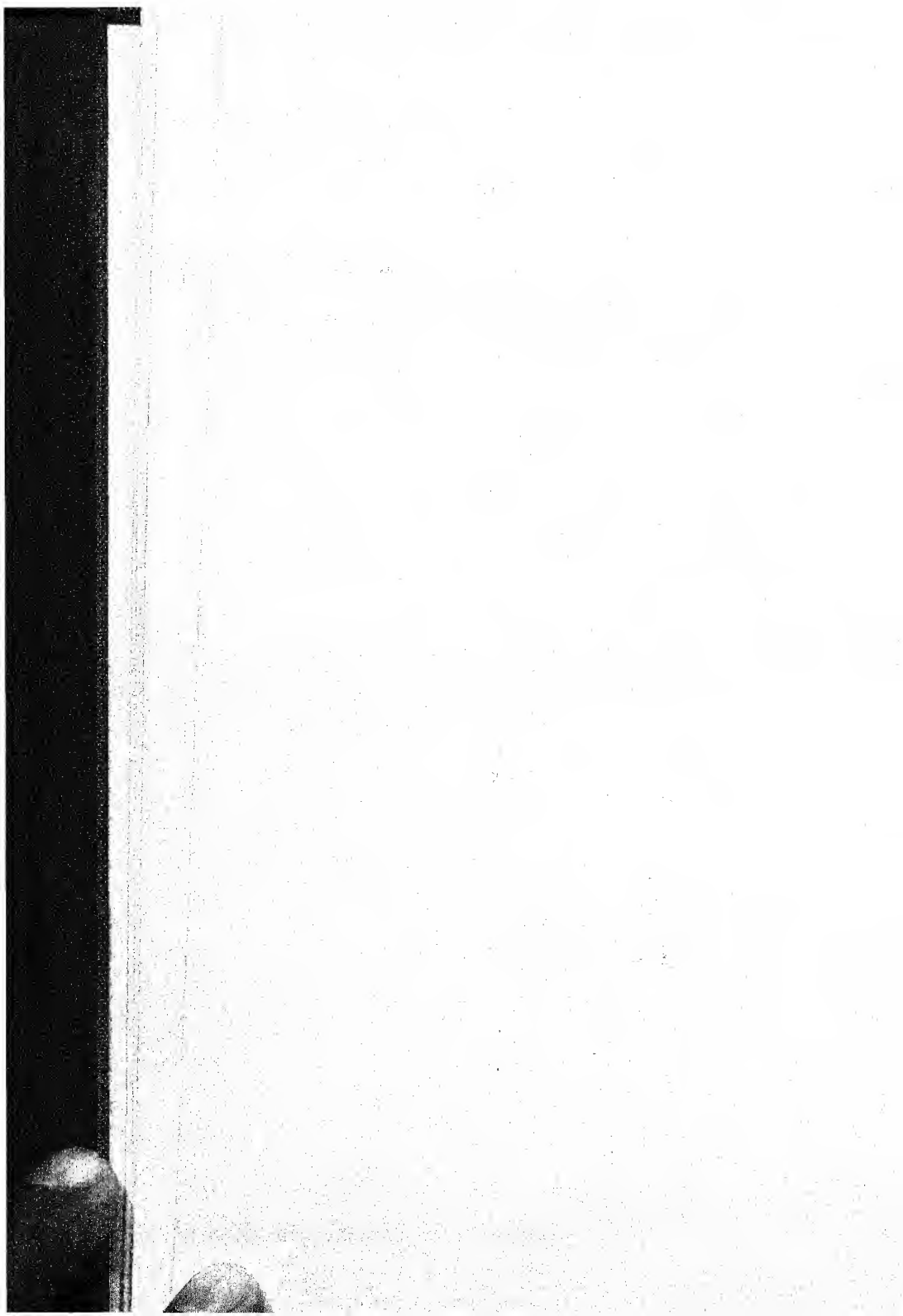<div align="right">A. J. B.</div>

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CHAPTER I

INTRODUCTION

General principles

Methods employed in quantitative chemical analysis may be divided broadly into two main classes, gravimetric and volumetric. In the former, the constituents of a substance are determined by separation and weighed in the form of compounds of known composition. In the latter, the substance to be estimated is allowed to react in solution with another substance of which a solution of known strength has been made; and the volume of the solution of known strength which is required for the completion of the reaction with a certain definite volume of the solution of the substance to be determined is observed. In order that a volumetric determination may be successfully carried out, it is essential that the end of the reaction may be clearly visible to the eye by the appearance or disappearance of some characteristic colour in the solution. Volumetric analysis possesses a great advantage over gravimetric analysis, viz., that the determination of a substance may be carried out with a very much smaller expenditure of time. In a gravimetric method it is necessary to separate the particular constituent which it is desired to determine in a state of great purity; while in a volumetric method such perfect separation is very seldom required, the presence of relatively large quantities of other substances which do not interfere with the particular reaction having in general no effect upon the accuracy of the determination.

Accuracy of volumetric analysis

The conditions which determine the accuracy of a volumetric method are threefold: firstly the purity of the substance which is employed for making up the solution of definite strength known as the standard solution; secondly the accuracy of the measuring

vessels; and thirdly, the sensitiveness of the change of colour or other device for indicating the completion of the reaction. When these three conditions are fulfilled, volumetric methods will be found to bear favourable comparison with the best gravimetric methods.
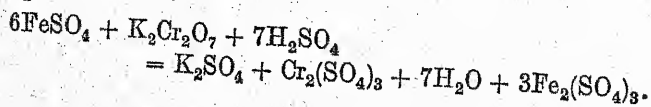
Equivalent weights

The equivalent weight of a substance is that weight of it which will react with a certain definite weight of some other substance. For example, it is an experimental fact that 169·9 grammes of silver nitrate will completely precipitate the chlorine in 74·56 grammes of potassium chloride by double decomposition according to the equation

$$AgNO_3 + KCl = AgCl + KNO_3.$$
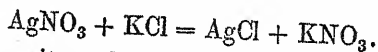
Again, if silver nitrate be precipitated by means of hydrochloric acid, it is found that 169·9 grammes of silver nitrate will precipitate the chlorine in 36·47 grammes of hydrogen chloride. These experimental results are expressed by saying that one gramme molecular weight of silver nitrate is equivalent to 74·56 grammes of potassium chloride and to 36·47 grammes of hydrogen chloride respectively.

Again, it has been shown by experiment that one gramme molecular weight (40·01 grammes) of sodium hydroxide is capable of neutralizing exactly 49·04 grammes of sulphuric acid, or 36·47 grammes of hydrochloric acid, or 45·01 grammes of anhydrous oxalic acid. In other words, these particular weights of sulphuric, hydrochloric and oxalic acids are said to be chemically equivalent to one gramme molecule of caustic soda and to one another.

If we consider a third type of reaction, viz., the oxidation of a solution of ferrous sulphate in presence of dilute sulphuric acid to ferric sulphate by means of potassium dichromate, accurate experimental work has shown that one gramme atomic weight of iron (metal), or 55·85 grammes, requires 49·03 grammes of potassium dichromate for complete transformation from the ferrous to the ferric condition, the reaction taking place in accordance with the equation

$$6FeSO_4 + K_2Cr_2O_7 + 7H_2SO_4$$
$$= K_2SO_4 + Cr_2(SO_4)_3 + 7H_2O + 3Fe_2(SO_4)_3.$$

The equivalent weight of potassium dichromate is therefore 49·03 relative to one gramme atomic weight of iron undergoing oxidation from the ferrous to the ferric condition.

It is clear from what has been stated that the equivalent weight of a compound is not necessarily identical with its molecular weight although it is closely related to it. In the case of an element the equivalent weight is equal to the quotient of the atomic weight by the valency. Considering only substances which combine directly with hydrogen, we might define the equivalent of a substance as that weight of it in grammes which will combine with 1·008 grammes of hydrogen. But if we wish to extend our definition in a consistent manner to substances which do not combine directly with hydrogen, we must study the behaviour of such substances towards some other element whose equivalent weight relative to hydrogen is accurately known. For example, it has been shown that 1·008 grammes of hydrogen combine directly with 35·46 grammes of chlorine producing 36·47 grammes of hydrogen chloride. If this gas be now dissolved in water it will be found to neutralize 40·01 grammes of sodium hydroxide according to the equation

$$HCl + NaOH = NaCl + H_2O.$$
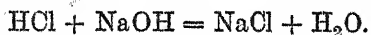
Again, the neutralization of sulphuric acid by caustic soda resulting in the formation of sodium sulphate and water takes place according to the equation

$$H_2SO_4 + 2NaOH = Na_2SO_4 + 2H_2O.$$
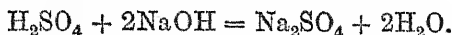
From this equation it follows that the equivalent weight of sulphuric acid is half its molecular weight. The double decomposition of hydrochloric acid and silver nitrate resulting in the formation of silver chloride and nitric acid shows that the equivalent of silver nitrate is 169·9.

In the third example which we have been considering, viz., the oxidation of a ferrous salt to the ferric condition by means of potassium dichromate, the value of the equivalent of this oxidizing agent is most easily seen from the fact that its decomposition may be regarded as due to the breaking down of the molecule $K_2Cr_2O_7$ into K_2O, Cr_2O_3, and three atoms of oxygen, which are effective in the conversion of the iron from the ferrous to the ferric condition.

Now it is an experimental fact that two molecules of hydrogen (four atoms) combine with one molecule (two atoms) of oxygen in the formation of water. Since the atomic weights of hydrogen and oxygen are respectively 1·008 and 16, it is clear that the equivalent of oxygen is 8, or in other words, one atom of oxygen is chemically equivalent to two atoms of hydrogen. Since potassium dichromate contains three atoms of oxygen which are available for the transformation of ferrous into ferric iron, these three atoms of oxygen are equivalent to six atoms of hydrogen. The equivalent weight of potassium dichromate is therefore one-sixth of its molecular weight or $\frac{1}{6}$ of 294·2 grammes or 49·03 grammes.

The three examples which we have discussed will indicate that by the adoption of equivalent weights a perfectly consistent inter-relationship between a large number of substances will be found to exist. We may therefore frame a definition of the term equivalent which will lead to such a relationship in the following terms. The equivalent of any substance, element or compound, is that weight of it in grammes which will either directly or indirectly bring one gramme of hydrogen into chemical action.

The importance of a consistent inter-relationship between the equivalent weights will be more readily apparent later. For the present the rôle of the equivalent in the calculation of results of volumetric determinations will be discussed.

Calculation of results

Suppose that a volume of v_1 cubic centimetres of a substance A is being determined, and that a volume of v_2 cubic centimetres of a substance B containing w grammes per cubic centimetre is required to complete the reaction, the weight x, in grammes, of substance A in each cubic centimetre is determined by the equation

$$\frac{v_1 x}{v_2 w} = \frac{\text{equivalent of } A}{\text{equivalent of } B} \quad \dots\dots\dots\dots(1).$$

Normal solutions

In volumetric analysis a considerable saving of arithmetical work may be effected by the employment of standard solutions which contain the equivalent weight of the substance in grammes dissolved in one litre of the solution. Such standard solutions

are termed normal solutions; or in other words, a normal solution may be defined as a solution of such a strength that one litre of it contains that weight of the solute which is chemically equivalent to one gramme of available hydrogen. For many purposes solutions of normal strength are too strong; in such cases, it is usual to employ solutions of semi-normal or of deci-normal strength, while for certain special work solutions of centi-normal strength are used. Solutions of normal, semi-normal, deci-normal, and centi-normal strength are conveniently abbreviated by the symbols $N, \frac{N}{2}, \frac{N}{10}, \frac{N}{100}$ respectively. The advantage of employing solutions of normal or of a sub-multiple of normal strength may be seen from the fact that 20 c.c. of a solution of deci-normal HCl will neutralize 20 c.c. of a deci-normal solution of NaOH or will precipitate 20 c.c. of a deci-normal solution of $AgNO_3$, in every case without any residue of either reagent remaining unacted on.

The reaction between equal volumes of solutions of normal or of some sub-multiple of normal strength however does not hold good in all cases, as the following example will show. Potassium bi-iodate $KH(IO_3)_2$ is a substance which can react either as an acid or as an oxidizing agent. In the former case, one molecule of the substance will neutralize one molecular proportion of potassium hydroxide with formation of two molecules of potassium iodate according to the equation

$$KH(IO_3)_2 + KOH = 2KIO_3 + H_2O.$$
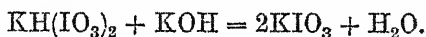
This reaction indicates that a normal solution of potassium bi-iodate should contain the molecular weight of the substance (390 grammes) dissolved in one litre. In the latter case potassium bi-iodate will liberate iodine from potassium iodide in presence of an acid, one molecule of the bi-iodate liberating six molecules of iodine as represented by the equation

$$KH(IO_3)_2 + 10KI + 11HCl = 6I_2 + 11KCl + 6H_2O.$$

Since one molecule of potassium bi-iodate liberates six molecules of iodine which are equivalent to twelve atoms of hydrogen, it follows that as an oxidizing agent the normal solution of this substance should contain one-twelfth of the molecular weight in grammes per litre.

It is clear, therefore, that the equivalent weight of a volumetric reagent is not an invariable magnitude like the molecular weight, but may be different in different reactions. It is in other words impossible to prepare normal solutions of *all* substances which shall possess the property that a given volume of one shall react quantitatively with an equal volume of any of the others. However, the number of substances which do conform consistently to the normal system is so great that even at the present time the system is of great practical value.

For many technical purposes it is usual to prepare standard solutions of such strength that the number of cubic centimetres of solution required for titration corresponds to a certain percentage of purity of the substance which is being examined.

Classification of methods in volumetric analysis

There are three main methods in volumetric analysis. The first is the direct method which includes all cases where the substance to be determined is estimated as the result of a single decomposition in the solution; such processes include the determination of acids by means of alkalis, of chlorides by silver nitrate, and of ferrous salts by means of potassium dichromate as well as numerous others. Secondly, there are indirect methods in which one or more intermediate reactions come into play; such processes include the determination of peroxides by distilling with hydrochloric acid, passing the chlorine into excess of potassium iodide, and determining the liberated iodine by means of sodium thiosulphate. Lastly, there are methods in which the substance to be determined is treated with a measured excess of some other substance for the purpose of reacting with it, and the excess of the added substance is then determined by some other reagent. As an example of this residual method, reference may be made to the estimation of ammonium salts by adding a known excess of standard alkali, boiling the solution to effect the decomposition of the ammonium salt and the removal of the ammonia, and then determining the excess of alkali remaining over by means of standard acid.

There are no general rules for selecting any one of these three general methods in preference to the others. There are many

substances which may be determined by any of these methods with equally satisfactory results. In certain cases it is convenient to make a combination of the direct or of the indirect method with the residual method.

Besides classifying volumetric methods according to the general experimental procedure, it is convenient to classify them according to the type of reaction which takes place in effecting the estimation. Three main types of chemical action are made use of in most of the commonly occurring volumetric processes. Firstly there is neutralization, or the double decomposition of acids and bases resulting in the formation of a salt and water; this method is employed for the estimation of acids and alkalis. In the second, analysis is effected by oxidation or reduction, the substance being converted from a lower to a higher degree of oxidation by means of an oxidizing agent of known value, or conversely from a higher to a lower degree of oxidation by means of a standard reducing agent. In the third the determination of the substance is effected by precipitation in an insoluble form by double decomposition, as in the determination of silver in solution by potassium chloride.

Classification of volumetric processes according to the main types of reaction which take place is convenient for many purposes, but it does not include all processes. The determination of copper by the decolorization of an ammoniacal solution of the metal by means of potassium cyanide does not admit of classification under any of the foregoing heads.

Apparatus employed in volumetric work

It is perhaps superfluous to give an account of the ordinary measuring vessels—pipettes, burettes, and measuring flasks— employed in volumetric analysis, since no written description of them can possibly make the reader as familiar with them as practical work in the laboratory. A few details regarding the use of burettes and pipettes however must be given. These vessels must be thoroughly cleansed before use, and then washed out with a small quantity of the liquid with which they are to be filled in order to prevent dilution of the solutions with water adhering

to the surface of the glass. The correct reading of graduated apparatus is important. In consequence of capillarity, the surface of a liquid in a narrow vessel is always curved; and if, as is usually the case, the liquid wets the glass, the surface will take the form of a concave meniscus. In reading the height of the liquid, the level of the lowest part of the meniscus is always taken, and it is important to place the eye on a level with the meniscus, as otherwise errors will be introduced. In allowing the contents of pipettes to flow into flasks previous to titration, sufficient time must be allowed for the liquid to drain down the walls of the vessel. The same remark may be made in titrating liquids when the liquid is allowed to flow rapidly from the burette; before reading the level of the liquid, time must be given for the liquid to drain down the tube, since otherwise the burette reading will be too high.

Errors in volumetric analysis

It has been already stated that the accuracy of a volumetric process depends on three main factors, viz., the purity of the substance employed for making up the standard solutions, the accuracy of the measuring vessels, and the sensitiveness of the change of colour for indicating the completion of the reaction. Besides these factors there are others which depend upon the conditions of experiment, and in this connexion reference must be made to the influence of the strength of the working solutions upon the accuracy of the process. This may perhaps best be illustrated by a practical example. Suppose it is desired to determine the percentage of sodium carbonate in a specimen of an impure alkaline substance by titration with standard hydrochloric acid. The reaction takes place in accordance with the equation

$$Na_2CO_3 + 2HCl = 2NaCl + H_2O + CO_2.$$
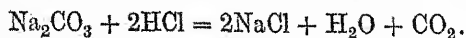
Is the determination more accurate when carried out with normal acid or with deci-normal acid?

Suppose that 0·5 gramme of the substance is weighed out and titrated with normal acid, and that 4·0 cubic centimetres of acid are required. The weight of anhydrous sodium carbonate is equal to $4 \times 0·053$ grammes, and the percentage of it is 42·4.

If in the determination an error of one-tenth of a cubic centimetre in the burette reading is made, that is, if 4·1 c.c. are used instead of 4·0 c.c., the weight of sodium carbonate is 4·1 × 0·053 grammes, and the percentage of it is 43·4.

If instead of weighing out 0·5 gramme for the determination, 5 grammes are taken and titrated with normal acid, 40 c.c. of acid will be required, corresponding to a weight of 40 × 0·053 grammes of sodium carbonate, or a percentage of 42·4.

Suppose that in the determination the reading of the burette is 40·1 c.c. instead of 40·0 c.c., the error introduced is only one part in 400, with the result that the percentage of sodium carbonate will be 42·5.

The result of the example which we are discussing is to show the advantage of adjusting matters so that a relatively large volume of liquid is run out from the burette. An error of one-tenth of a cubic centimetre gives rise to a smaller error the greater the volume of the liquid run out.

If we modify the procedure in the above experiment by titrating the alkaline carbonate with deci-normal hydrochloric acid instead of the normal acid, and take 0·5 gramme of the substance, the titration with deci-normal hydrochloric acid will require 40 c.c., corresponding to 40 × 0·0053 grammes of sodium carbonate, that is to a percentage of 42·4. If an error of 0·1 c.c. is made, that is, if the burette reading is 40·1 c.c. instead of 40·0 c.c., the error in the final result will lead to a percentage of sodium carbonate equal to 42·5, or approximately to an error of one part in four hundred.

It is clear that we have realized the same degree of accuracy by working with deci-normal acid and taking the smaller quantity of substance as we have realized by employing normal acid with ten times the quantity of substance. When economy of material is a consideration, the employment of the more dilute acid is therefore to be recommended.

The advantage of working with a deci-normal standard solution in preference to one of normal strength is however obviously only secured if the change of colour denoting the completion of the reaction, or the end-point as it is called, is defined with equally great precision in dilute solution to what it is in a more concentrated solution. This is by no means always the case; and in the

particular example which has been quoted, where the end point is usually defined by the addition of a drop of methyl orange (Chapter VIII), the change of colour is somewhat more sharply defined in solutions of normal strength than in solutions of deci-normal strength. For this reason, it is inadvisable to frame any hard and fast rules for the employment of more dilute rather than of more concentrated standard solutions; all that can be said is that the conditions should be adjusted so that as large a volume of liquid is allowed to flow from the burette as possible; in no case should less than 10 c.c. be run in.

In discussing the accuracy of volumetric determinations, particularly as regards the calculation of results from experiments, it is of course essential to know how much reliance is to be placed upon the measuring vessels. Ordinary pipettes and burettes are usually to be relied upon to an accuracy of the order of one-half per cent. If greater accuracy than this is required it is necessary to calibrate the measuring vessels by filling with water and deter-mining the weight of water which they contain or deliver. If we are dealing with ordinary apparatus which has not been specially calibrated, it is not only mere waste of time to continue the arith-metical work beyond a certain point, but the result has no definite meaning. The process of calculation should be carried only a *little* way beyond the degree of accuracy of the experimental work, in order to ensure that no error is introduced into the final result by the process of calculation.

In connexion with the accuracy of measuring vessels, it is important to bear in mind that the process of weighing out the substance for the standard solution need not be carried out with greater precision than corresponds to the accuracy of the vessels which are to be employed to measure the standard solution. As a matter of fact the process of weighing should be carried out a *little* more accurately than the accuracy of the measuring vessels in order to ensure that the maximum accuracy of the standard solution is realized.

In carrying out volumetric determinations, it is sometimes asked by beginners whether to run the standard solution from the burette into the solution the strength of which is being determined, or to reverse the process, that is, to measure out a known volume

of the standard solution and titrate with the solution which is under investigation. It is obvious that if both measuring vessels are equally accurate, and if the change of colour denoting the end-point of the reaction is defined with equal precision in either direction, the result should be identical whichever way the titration is carried out. From that point of view it is immaterial whether the standard solution is used in the pipette or in the burette; nevertheless it will in most cases be found more convenient to run the standard solution from the burette into the unknown solution. In cases, however, in which the colour change is defined with greater precision by reversing the usual procedure, the advantage of greater accuracy should be secured. This is particularly to be observed in cases in which the strength of the standard solution has been determined under special conditions which require to be uniformly satisfied in order to obtain consistent results, as is the case with some volumetric processes which are purely empirical.

It cannot be too strongly impressed upon students that in volumetric analysis, as in all other quantitative experimental work, it is a fatal mistake to be satisfied with a single observation. The student should make it a rule for himself never to calculate the result of a determination from a single titration. Two or three concordant titrations should always be obtained before proceeding to the calculation, which is to be carried out with "approximate" atomic weights. "Accurate" atomic weights are only to be used when carefully calibrated measuring vessels are employed in the experiments.

Preparation and storage of standard solutions

Solutions of known strength may readily be prepared by dissolving the weighed quantity of the pure substance in water and diluting to the necessary volume. When large quantities of standard solution are required, and when the purity of the dissolved substance is open to doubt, solutions of approximate strength may be prepared and the exact strength determined by titration with a solution of accurately known strength prepared from the purest materials. In all cases of making up large quantities of standard solutions from substances which are only approximately pure, it

is advisable to take a slightly greater quantity of substance than the theoretical, in order that a solution of the required strength may be obtained by dilution after the exact strength has been determined by titration. If solutions of normal or sub-normal strength are required and a solution of a strength approximating closely to the exact value has been prepared, it is often more

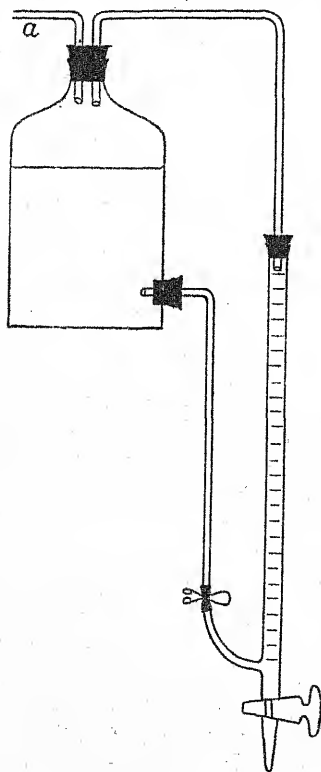Fig. 1. Reservoir in direct communication with burette. The tube *a* may be placed in direct connexion with an apparatus for generating hydrogen.
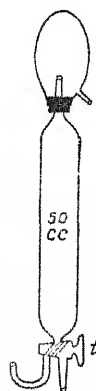
Fig. 2. Automatic delivery pipette. The apparatus is filled or emptied by the tap *t*.

convenient to determine by titration how far the solution deviates from the strictly normal or sub-normal strength, and to express the deviation in terms of a factor, than to dilute by adding more

water, or to concentrate by evaporation to make the strength of
the solution the exact value. For many purposes, however, it is
more convenient to work with solutions the exact strength of which
is known and to calculate the results of the analyses from the
general equation (p. 4)

$$\frac{v_1 x}{v_2 w} = \frac{\text{equivalent of } A}{\text{equivalent of } B}.$$

The permanence of standard solutions varies greatly with the
nature of the dissolved substance. Some substances, like potas-
sium dichromate, are absolutely permanent in aqueous solution,
so that if precautions are taken to guard against loss of solvent
by evaporation, the strength of the solutions will remain unchanged
for an indefinite period. Other substances, like sodium thio-
sulphate, are much less stable in solution, so that the standard
solutions should be titrated at frequent intervals in order to verify
their exact strength. Some substances like potassium perman-
ganate are somewhat sensitive to light; solutions of such substances
should therefore be preserved in dark blue bottles and kept in
the dark.

For many purposes, when the standard solutions require to be
protected from the air, as is the case with titanous chloride, it is
convenient to store the solution in a reservoir which is in direct
communication with a burette (Fig. 1) or with an automatic
delivery pipette (Fig. 2).

The thermal expansion of solutions

In the preparation of standard solutions, the influence of
temperature on the volume of the resulting liquid must be carefully
borne in mind. The coefficient of expansion of solutions is by no
means identical with that of pure water; in general, solutions
possess a higher coefficient. Consequently if heat has been em-
ployed in dissolving a solid for the preparation of a standard
solution, sufficient time must be allowed for the liquid to acquire
a constant volume before diluting it to a standard volume. The
same remark applies to the case of substances which dissolve in
water with considerable absorption or evolution of heat. The
temperature coefficient of expansion of solutions of moderate

strength is in general considerably greater than that of more dilute solutions. This is an additional reason for preferring solutions of the order of deci-normal strength to those of normal strength.

In connexion with the thermal expansion of liquids, mention may be made of the degree of accuracy with which burettes should be read. The burettes in common use are graduated in tenths of a cubic centimetre, and may be read with accuracy to one-twentieth. Even in the case of burettes which are calibrated accurately, it is mere waste of time to attempt to read the volume of liquid to any greater degree of accuracy than this, unless the temperature of the liquid is maintained within narrow limits.

Reactions in aqueous solution

When a substance is dissolved in water, the properties of the resulting solution indicate that in many cases a change takes place. It is by no means always an easy matter to say whether the change is to be regarded as a physical or a chemical one. In some cases it appears that the substance dissolves without change. That is to say, the properties of the solution depending upon osmotic effects indicate that the substance in solution possesses a molecular weight equal to that which is arrived at by other methods. Alcohol and cane sugar dissolve to form solutions of this kind. Again, hydration may take place. Hydration is a true chemical change, the substance actually uniting with one or more molecules of water when brought in contact with the solvent. Further, hydrolysis may take place. It may be stated that any reaction in which water plays an essential part other than by mere addition is termed hydrolysis. This phenomenon is of exceedingly common occurrence and manifests itself more particularly in connexion with the appearance of acidic or basic properties when certain salts are dissolved in water. It is of great importance in reactions involving neutralization. Lastly, the properties of solutions of that large class of substances known as salts are of such a nature that the theory has been put forward that these substances undergo electrolytic dissociation to a greater or less extent when dissolved in water. Solutions of substances which are electrolytically

dissociated are extremely reactive, and the reactions are so rapid as to be practically instantaneous. Such reactions play a very prominent part in volumetric analysis; but for most purposes it is unnecessary to introduce the ionic theory in the discussion of volumetric methods, and it is perhaps preferable to avoid doing so. The theory of indicators (Chapter IX) is however so very greatly facilitated by the hypothesis of ionization that no apology is necessary for making free use of it in that connexion.

Most of the reactions employed in volumetric analysis are capable of giving satisfactory results under a wide range of experimental conditions, such as the degree of dilution of the solutions and the presence or absence of inert substances. Nevertheless, in order to realize the maximum accuracy of any particular volumetric process, it is always desirable, and in some cases important, to standardize the solutions under conditions as nearly identical as possible with those under which they are to be employed.

CHAPTER II

DETERMINATIONS WITH STANDARD POTASSIUM PERMANGANATE

Potassium permanganate, $KMnO_4$, is a dark crystalline solid, which dissolves in water forming a beautiful deep purple solution. This solution possesses very powerful oxidizing properties. Its maximum oxidizing action is obtained when it is employed in presence of free acid. For this purpose sulphuric acid is employed. Nitric acid being itself possessed of oxidizing properties is used only in exceptional cases. Hydrochloric acid has been employed in certain cases, but the use of this acid is not to be recommended, for reasons which we shall explain later. In order to understand the oxidizing power of potassium permanganate in presence of sulphuric acid, it is necessary to bear in mind that the essential action consists in the decomposition of the permanganate in accordance with the equation

$$2KMnO_4 + 3H_2SO_4 = K_2SO_4 + 2MnSO_4 + 3H_2O + 5O.$$
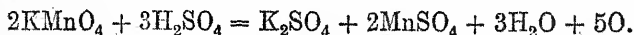
The five atoms of oxygen are not liberated in the gaseous state but are given up to any oxidizable substance which may be present in the solution. Thus oxalic acid is converted into carbon dioxide and water

$$5H_2C_2O_4 + 5O = 10CO_2 + 5H_2O.$$
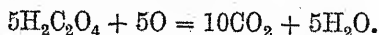
Again, we may represent the oxidation of a ferrous salt as consisting in the conversion of ferrous oxide FeO into ferric oxide Fe_2O_3 as follows

$$10FeO + 5O = 5Fe_2O_3.$$
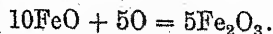
The complete equation for the oxidation of oxalic acid is therefore

$$2KMnO_4 + 3H_2SO_4 + 5H_2C_2O_4$$
$$= K_2SO_4 + 2MnSO_4 + 8H_2O + 10CO_2.$$
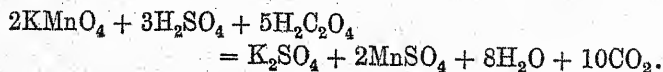
Again, the complete equation representing the conversion of a ferrous salt into a ferric salt may be written

$$2KMnO_4 + 8H_2SO_4 + 10FeSO_4$$
$$= K_2SO_4 + 2MnSO_4 + 8H_2O + 5Fe_2(SO_4)_3.$$

Many other substances may be oxidized quantitatively by potassium permanganate in presence of sulphuric acid. We shall discuss these later.

We defined a normal solution of a volumetric reagent as a solution containing in one litre that fraction of the molecular weight which corresponds to one gramme of available hydrogen. Now in the case of pot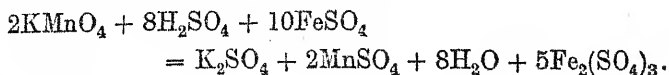assium permanganate, since two molecules of $KMnO_4$ give rise to five atoms of available oxygen, and since five atoms of oxygen are equivalent to ten atoms of hydrogen, the normal solution of this substance must contain one-fifth of the molecular weight in grammes in one litre. In other words, the normal solution should contain 31·6 grammes of the salt per litre. It is however more usual to employ this reagent of deci-normal strength, the solution containing therefore 3·16 grammes of the substance dissolved in one litre.

Potassium permanganate is now readily obtainable of a very fair degree of purity. A solution of approximately deci-normal strength may therefore be readily prepared by dissolving the requisite quantity of the substance in water and diluting the solution to the calculated volume. It is necessary to determine the exact strength of the solution; and this may be done in either of the two following ways.

(a) *By ferrous ammonium sulphate*, $FeSO_4(NH_4)_2SO_46H_2O$. This double salt may be prepared in a high degree of purity by recrystallization of the commercial product. Or the salt may be prepared by dissolving in separate small quantities of hot water amounts of ferrous sulphate, $FeSO_47H_2O$, and of ammonium sulphate, $(NH_4)_2SO_4$, in the proportions of their respective molecular weights. It is advisable to add a few drops of dilute sulphuric acid to the iron solution to prevent the formation of basic salt. On mixing the two solutions and cooling, the double salt, being less soluble in water than the single salts, separates in the form of small crystals. These crystals are washed with cold water,

recrystallized, and finally dried by pressing between filter paper. A suitable quantity, say 4 or 5 grammes, is then weighed out carefully, dissolved in water acidified with dilute sulphuric acid and made up to some definite volume. Aliquot portions are then withdrawn and the solutions titrated with the solution of potassium permanganate. The permanganate is added from a burette provided with a glass tap: a rubber joint must not be used as it would reduce the permanganate. No indicator is added to the liquid, since the slightest excess of permanganate imparts a permanent pink colour to the solution. Several titrations must be made, and they must agree with one another to one-half per cent. The double iron salt contains almost exactly one-seventh of its weight of iron, as may be seen by calculating its percentage composition from its molecular formula.

If v_1 c.c. of the ferrous solution require for complete oxidation v_2 c.c. of $KMnO_4$, the strength of the solution of potassium permanganate is given by the equation

$$\frac{v_1 \times \text{weight of iron in 1 c.c.}}{v_2 x} = \frac{\text{equivalent of iron}}{\text{equivalent of permanganate}} = \frac{10 \times 56}{2 \times 158},$$

where x is the weight of the permanganate per c.c.

(b) *By oxalic acid.* As we have mentioned already, oxalic acid is oxidized quantitatively to carbon dioxide and water by potassium permanganate in presence of dilute sulphuric acid. The recrystallized acid containing two molecules of water of crystallization serves admirably therefore for the standardization of permanganate. Some operators prefer to use sodium oxalate, $Na_2C_2O_4$, which crystallizes anhydrous, as there seems to be a slight doubt about the constancy of the water of crystallization of the free acid. In either case the method of procedure is the same. A standard solution of oxalic acid or of sodium oxalate is prepared by dissolving a weighed quantity of the substance in water and diluting the solution to the required volume. Aliquot portions are then withdrawn for the titration, acidified with dilute sulphuric acid, and warmed to about 60° or 70° C. and the permanganate solution added slowly until a faint permanent pink colour remains in the liquid. The reason for warming the solutions previous to titration is that the velocity of the reaction between oxalic acid

and potassium permanganate is not great at the ordinary temperature, but as is usually the case, the reaction velocity is enormously increased by an elevation of temperature.   Once the reaction has started, the manganous sulphate, which is formed as a by-product of the reaction, acts as a catalyst on the oxidation of the oxalic acid. Oxalic acid may indeed be titrated at the ordinary temperature if a quantity of manganous sulphate is added at the beginning, but the process is in any case considerably slower than if the solution be warmed. The reaction between oxalic acid and potassium permanganate in presence of sulphuric acid is an interesting example of autocatalysis, *i.e.* of catalysis of the main reaction by one of the products of the reaction.

The calculation of the strength of the solution of potassium permanganate is made from the equation

$$\frac{v_1 \times (\text{weight of oxalic acid per c.c.})}{v_2 x}$$
$$= \frac{\text{equivalent of oxalic acid}}{\text{equivalent of permanganate}} = \frac{5 \times 126}{2 \times 158}.$$

In titrating oxalic acid or a ferrous salt by permanganate it is important not to add the reagent too rapidly, otherwise a brown precipitate of hydrated higher oxides of manganese may be produced, and it is exceedingly difficult to remove this precipitate afterwards.   All titrations in which a precipitate appears should be rejected.

A standard solution of potassium permanganate will retain its strength for a long period, especially if preserved from light by storing it in dark blue bottles.

Determination of ferrous and ferric iron by potassium permanganate

The determination of ferrous salts has virtually been described in discussing the use of ferrous ammonium sulphate as a reagent for standardizing permanganate, and no further discussion is necessary.   Ferric salts must first be reduced to the ferrous condition, and the resulting solution titrated by permanganate in the ordinary way.   Of the various substances employed as reducing agents, the following are the most generally used: zinc and sulphuric acid, hydrogen sulphide, and sulphur dioxide.

The procedure for reducing ferric solutions by means of zinc and sulphuric acid is as follows. Aliquot portions of the iron solution are placed in separate flasks, a moderate amount of granulated zinc and dilute sulphuric acid added to each and the contents warmed. From time to time a drop of the liquid undergoing reduction is removed by means of a glass rod and brought in contact with a drop of ammonium thiocyanate on a white tile. As long as any ferric iron remains, a dark red colour is produced due to the formation of ferric thiocyanate, but when the iron is completely converted to the ferrous condition, the drops on the spot plate remain colourless when tested with the iron solution. The solution of ferrous sulphate is next separated from undissolved zinc by filtration through glass wool. Water is next added to the flask, allowed to flow through the glass wool, and the washings added to the reduced filtrate, which is titrated with potassium permanganate in the usual way.

The reduction of ferric salts to the ferrous condition by means of zinc and dilute sulphuric acid is frequently regarded as taking place in consequence of the so-called nascent hydrogen generated by the action of the metal on the acid, since it is impossible to effect the reduction of the ferric salt by simply bubbling hydrogen through the solution. It must be pointed out, however, that no such explanation can be regarded as satisfactory, whatever views are entertained with regard to the nascent state. If there be any truth in the idea of nascent hydrogen, it is obvious that this substance should possess the same properties whatever be the source from which it is derived. For example, some compounds can be reduced by hydrogen generated by the action of sodium amalgam in presence of water, while others require the gas to be generated in an acid solution by the action of metals such as tin or zinc. It has been definitely established by experiments on electrolytic reduction that the reducing power of hydrogen is connected with the potential, and for these reasons it is no longer accurate to speak of 'nascent' hydrogen as if it were a chemical individual.

In reducing a solution of ferric sulphate to the ferrous condition by means of zinc and sulphuric acid, it is advisable in all cases to follow the method of procedure already described, viz., to add a considerable excess of granulated zinc and to separate the excess

of the metal by filtration after the reduction of the iron solution is complete, rather than to add only a small quantity of zinc and allow the action to continue until all the metal has dissolved, because the reduction takes place essentially at the surface of the zinc, and the process is all the more rapid the greater the surface of metal exposed to the solution. The reduction of ferric salts by means of zinc yields excellent results. It is perhaps scarcely necessary to point out that the zinc used must be free from iron and other impurities such as arsenic.

If sulphuretted hydrogen be selected as the reducing agent, aliquot portions of the iron solution are diluted with water, acidified with a little dilute sulphuric acid, and saturated with the gas

$$Fe_2(SO_4)_3 + H_2S = 2FeSO_4 + H_2SO_4 + S.$$
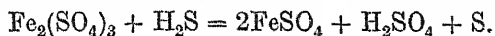
When it has been ascertained by the thiocyanate test that reduction is complete, the solution is boiled to expel the excess of hydrogen sulphide, and to granulate the precipitate of sulphur. The sulphur is then filtered off, washed with water, and the filtrate and washings titrated with permanganate.

For many purposes the reduction of solutions of ferric iron may be effected in a very satisfactory manner by the use of sulphur dioxide. The gas may be generated *in situ* by adding a few crystals of sodium sulphite to the measured quantity of the solution of the ferric salt, and acidifying with dilute sulphuric acid; or the solution may be saturated directly with sulphur dioxide by bubbling the gas from one of the syphons of the liquid. In either case it is important to have the solution dilute and to avoid having too much free acid present, otherwise the reduction does not proceed very easily

$$Fe_2(SO_4)_3 + SO_2 + 2H_2O = 2FeSO_4 + 2H_2SO_4.$$
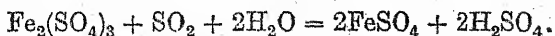
The solution is now heated to boiling and drops are brought in contact with drops of ammonium thiocyanate in order to observe the progress of the reaction. The boiling is continued until the sulphur dioxide is completely expelled. The solution is then cooled and titrated with permanganate.

It is to be observed that while all three reduction methods give satisfactory results as far as the actual reduction of a ferric solution is concerned, circumstances may arise when one or other method

is to be preferred. For example, if for any reason it is essential to avoid the introduction of a foreign metal into the solution, then one would make use of hydrogen sulphide or sulphur dioxide as the reducing agent.

Determination of ferrous and ferric iron in a mixture

Measured quantities of the solution are acidified with dilute sulphuric acid, and then titrated with potassium permanganate in the usual way. If n_1 c.c. of permanganate are required, this quantity represents the amount of ferrous iron in the quantity of the mixture taken. The same quantities of the mixture are again measured out and reduced by any of the methods already described. These solutions are then titrated with permanganate. This second titration, n_2 c.c., represents the total iron in the measured quantity of the solution. The ferric iron is therefore represented by $(n_2 - n_1)$ c.c. of potassium permanganate.

### Determination of hydrogen peroxide in aqueous solution

Since hydrogen peroxide and potassium permanganate react in presence of sulphuric acid in the manner represented by the equation

$$5H_2O_2 + 2KMnO_4 + 3H_2SO_4 = K_2SO_4 + 2MnSO_4 + 8H_2O + 5O_2,$$

the former substance may be very easily determined. But since commercial hydrogen peroxide frequently contains organic matter to act as a preservative to prevent spontaneous decomposition, this organic matter undergoes oxidation by permanganate, and the method therefore is liable to give high results. Consequently this method is not to be recommended for the determination of this substance. A better method of determining hydrogen peroxide is described in Chapter v.

Determination of ferrocyanides

Potassium ferrocyanide is very easily oxidized to potassium ferricyanide by means of potassium permanganate in presence of dilute sulphuric acid. The reaction takes place in accordance with the equation

$$10K_4FeC_6N_6 + 8H_2SO_4 + 2KMnO_4$$
$$= 10K_3FeC_6N_6 + 6K_2SO_4 + 2MnSO_4 + 8H_2O.$$
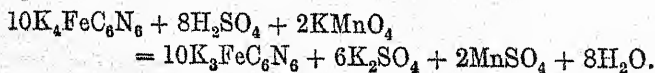
In presence of dilute sulphuric acid, the ferrocyanide solution possesses a greenish yellow colour. The ferricyanide on the other hand is possessed of a golden yellow colour. In this reaction the end-point to be aimed at is not the faint pink colour to which the operator is familiar in permanganate work, but the point at which the solution changes from greenish yellow to a rich golden brown. A little practice will soon enable the experimenter to determine the point without difficulty.

Determination of oxidizing agents by means of potassium permanganate

Many oxidizing agents may be determined in a very satisfactory manner by allowing them to react with a known excess of ferrous sulphate, and then determining by titration with potassium permanganate the amount of ferrous iron remaining. The difference between the amount of ferrous iron originally taken and that found by the permanganate titration represents the oxidizing power of the substance which is being determined. Persulphates, for example, may be determined in this way.

Use of potassium permanganate in presence of hydrochloric acid

The simplicity and accuracy of the use of permanganate has led many chemists to attempt to employ it as a volumetric process in presence of hydrochloric acid instead of sulphuric acid in cases where the use of the former acid is necessary. But, unless certain precautions are taken, the titration of ferrous iron by means of potassium permanganate in presence of hydrochloric acid gives rise to inaccurate results. It has been stated by some chemists that hydrochloric acid and potassium permanganate react together with evolution of chlorine according to the equation

$$2KMnO_4 + 16HCl = 2KCl + 8H_2O + 2MnCl_2 + 5Cl_2,$$
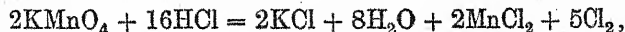
and that complications are thereby introduced. It is, however, certain that the formation of chlorine does not take place in *dilute* solutions in the simple manner represented by this equation, since it is impossible to demonstrate the formation of chlorine by merely bringing together *dilute* solutions of hydrochloric acid and

potassium permanganate.   But if dilute solutions of hydrochloric
acid and potassium permanganate are brought together in presence
of a ferrous salt, then chlorine is formed in sufficiently great amount
to be easily recognizable by its odour; in other words, the evolution
of chlorine is in some manner connected with the presence of iron.
It has further been shown by Fresenius that if one takes a given
volume of a hydrochloric acid solution of a ferrous salt, and titrates
it with potassium permanganate, and then adds to the titrated
solution an equal volume of the original iron solution, and again
titrates, the volume of permanganate required to complete the
reaction is greater in the first case than in the second.   If the
experiment be repeated a third and a fourth time in the manner
described, it will be found that the volumes of permanganate will
become still smaller, and then remain constant.   When constant
volumes of permanganate are obtained, it will be found that those
volumes are an approximately correct measure of the amount of
ferrous iron which is undergoing oxidation.   It has also been found
that approximately correct results are obtained if a moderate
quantity of manganous sulphate be added to a hydrochloric acid
solution previous to titration with potassium permanganate.

We have now to ask ourselves two questions; first, in what
manner does iron act in causing the formation of chlorine from
a mixture of dilute solutions of hydrochloric acid and potassium
permanganate, and, secondly, how does the addition of manganous
sulphate to the solution prevent the formation of chlorine?   It
would scarcely be within the scope of the present work to enter
into an elaborate discussion of the various explanations which
have been offered for these curious phenomena, but it may be stated
that the theory which has perhaps attracted most attention is
that developed by Manchot and others, according to which the
iron while being converted from the condition corresponding to
ferrous oxide, FeO, absorbs oxygen so rapidly from the perman-
ganate that a higher oxide of iron than ferric oxide, Fe_2O_3, is
formed, perhaps Fe_2O_5.   This higher oxide then decomposes into
ferric oxide and oxygen, which reacts with the hydrochloric acid
with development of chlorine.   If, however, a manganous salt is
present in solution, this salt is capable of acting as a carrier of
oxygen, the manganous salt being oxidized by the iron peroxide

to a higher oxide of manganese, and this higher oxide of manganese effects the oxidation of more ferrous iron.

The oxidation of a ferrous salt by acidified potassium permanganate may be represented in the following manner

$$Mn_2O_7 + 2FeO = 2MnO_2 + Fe_2O_5 \quad \dots\dots(1),$$
$$Fe_2O_5 + 4FeO = 3Fe_2O_3 \quad \dots\dots\dots\dots(2).$$
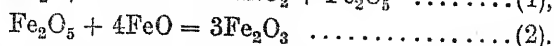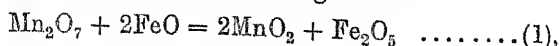
The manganese dioxide formed according to equation (1) also reacts with more ferrous oxide, resulting in the formation of ferric oxide

$$2MnO_2 + 4FeO = 2Fe_2O_3 + 2MnO \quad \dots\dots(3),$$
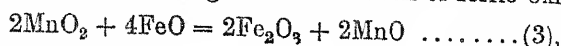
so that the familiar result of ten molecules of ferrous salt becoming oxidized by two molecules of permanganate into five molecules of ferric salt is obtained. Reaction (2), according to Manchot (*Annalen der Chem.* **325**, 1902, p. 105), takes place in sulphuric acid solution with such great rapidity that no free oxygen is liberated, and consequently the results are accurate.

In presence of hydrochloric acid, however, the iron peroxide reacts with the hydrochloric acid resulting in the formation of chlorine, according to the equation

$$Fe_2O_5 + 10HCl = 2FeCl_3 + 5H_2O + 2Cl_2 \quad \dots\dots(4).$$
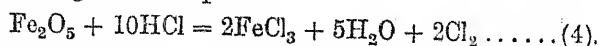
But, if a manganous salt is present in sufficient quantity, the production of chlorine is prevented in two ways, first, because the manganous salt reacts with the permanganate forming hydrated manganese dioxide according to the equation

$$Mn_2O_7 + 3MnO = 5MnO_2 \quad \dots\dots\dots(5),$$
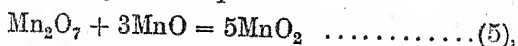
and, secondly, because the manganous oxide reacts with the iron peroxide resulting in the formation of ferric oxide and manganese dioxide

$$Fe_2O_5 + 2MnO = 2MnO_2 + Fe_2O_3 \quad \dots\dots(6).$$
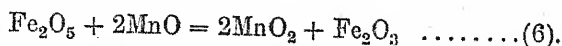
This explanation of the formation of chlorine during the titration of a hydrochloric acid solution by means of potassium permanganate, and the prevention of the evolution of chlorine by the addition of a sufficient quantity of a manganous salt, seems to accord fairly well with the observed facts; but, nevertheless, the titration of iron by potassium permanganate in presence of hydrochloric acid is not to be recommended, as undoubted irregularities have been found to occur even in presence of excess of a manganous

salt. The method is at best an empirical one. In particular, it has been shown by Birch (*Chem. News*, 1909, pp. 61 and 73) that the method of conducting the determination as recommended by Fresenius does not give accurate results. If it is necessary to determine iron in presence of hydrochloric acid, the best plan is to employ potassium dichromate as the oxidizing agent.

Determination of metals which form insoluble oxalates

Inasmuch as oxalic acid may be determined by permanganate with great ease and accuracy, metals which are quantitatively precipitated as oxalates may be estimated by decomposition of the oxalate by means of dilute sulphuric acid, and titration of the resulting oxalic acid by standard potassium permanganate. As an example we shall describe the procedure for the determination of calcium in Iceland spar ($CaCO_3$).

A suitable quantity of Iceland spar is weighed out and dissolved in excess of dilute hydrochloric acid, care being taken to avoid loss of liquid by spirting. The solution is next made alkaline with ammonia, a small quantity of ammonium chloride added, and the calcium precipitated by addition of excess of ammonium oxalate. The precipitate is next collected, washed free of the excess of the precipitant, and then decomposed with dilute sulphuric acid. The solution is next made up to a known volume with distilled water, and aliquot portions are titrated with potassium permanganate.

Since two molecules of $KMnO_4$ are equivalent to five molecules of oxalic acid, this quantity of permanganate is equivalent to five atoms of calcium.

It is clear that the process is applicable to any metal which forms an insoluble oxalate. The process may be modified by precipitating the metal with a known excess of a soluble oxalate, and determining by means of permanganate the quantity of oxalate which remains unprecipitated. This latter method, however, does not possess any advantage over that already described.

Ferrous oxalate is analysed by double titration with permanganate. In acid solution the iron is oxidized to the ferric state and the oxalate is decomposed. The solution is then reduced completely and the iron again oxidized with permanganate.
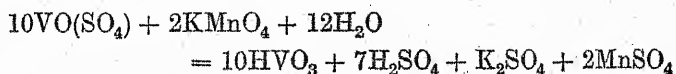
Determination of vanadium

Vanadium exists in four states of oxidation which correspond to the oxides V_2O_2, V_2O_3, V_2O_4, and V_2O_5. The ions corresponding to these oxides have distinctive colours. The vanadous salts corresponding to the oxide V_2O_2 have a violet colour. The vanadic salts derived from the oxide V_2O_3 are green. In the vanadous salts the element is bivalent, in the vanadic salts it is tervalent. The tetroxide V_2O_4 gives rise to salts which have a fine blue colour corresponding to the complex bivalent vanadyl ion $\overset{++}{V}O$, such as the sulphate $VO(SO_4)$. The oxide V_2O_5 is wholly acidic and is the anhydride of vanadic acid, the chemistry of which is complicated. When ammonium metavanadate, NH_4VO_3, is ignited, the pentoxide is obtained as an orange red powder, very sparingly soluble in water to a pale yellow solution with an acid reaction to indicators. It is more readily soluble in alkalis than in acids. Solutions of vanadates rarely contain the simple $\overset{-}{V}O_3$ anion, more complex anions are usually present.

Reduction of vanadates to vanadous salts may be effected in acid solution with zinc. This is most conveniently effected with zinc amalgam (see page 161), reduction being followed by characteristic colour changes from yellow to blue, then to green, and finally to violet. Milder reducing agents, such as ferrous sulphate, sulphur dioxide, or hydrogen sulphide, convert vanadates into the blue vanadyl salts, but further reduction does not take place. Potassium permanganate in presence of dilute sulphuric acid will oxidize vanadium in any of the lower stages ultimately to vanadic acid. The volumetric determination of the element is based on these reactions.

On account of the great sensitiveness of the vanadous salts to atmospheric oxidation, it is best to obtain the vanadium in the vanadyl condition before oxidizing with potassium permanganate. The vanadyl salts are much more stable, and are quantitatively oxidized by potassium permanganate if the liquid is gently warmed towards the end of the reaction:

$$10VO(SO_4) + 2KMnO_4 + 12H_2O$$
$$= 10HVO_3 + 7H_2SO_4 + K_2SO_4 + 2MnSO_4$$

or more simply, $V_2O_4 + O = V_2O_5.$

*Determination of copper by reduction to cuprous oxide and
subsequent determination by ferric sulphate and permanganate*

If excess of sodium hydroxide be added to a solution of a copper
salt, a blue precipitate of hydrated copper oxide is obtained.
This precipitate is readily soluble in solutions of certain organic
compounds containing hydroxyl groups, such as tartaric acid.
From a solution of cupric oxide in an alkaline tartrate, reducing
agents such as glucose will readily precipitate cuprous oxide, Cu_2O,
as a red powder on warming. The precipitation is quantitative,
and the metal is readily determined by separating the cuprous
oxide and dissolving it in a solution of a mixture of ferric sulphate
and sulphuric acid, when reduction of the iron salt occurs in
accordance with the equation

$$Cu_2O + H_2SO_4 + Fe_2(SO_4)_3 = 2CuSO_4 + 2FeSO_4 + H_2O.$$
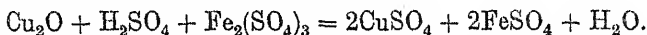
The resulting ferrous sulphate is then determined by titration
with potassium permanganate in the ordinary manner.

In carrying out a determination of copper by this method it
is unnecessary for the metal to be present as cupritartrate, and
hydroxylamine will be found to be a better reducing agent than
glucose. A solution of hydroxylamine hydrochloride is added in
slight excess, together with excess of aqueous sodium hydroxide to
measured quantities of the solution of the cupric salt. Reduction
takes place rapidly on heating with separation of cuprous oxide,
the hydroxylamine being oxidized to nitrous oxide, thus

$$4CuO + 2NH_2OH = 2Cu_2O + N_2O + 3H_2O.$$
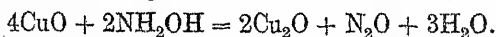
The precipitate is then filtered off, washed thoroughly with water,
and dissolved in a solution of ferric sulphate acidified with sulphuric
acid. The amount of copper is then determined by the perman-
ganate titration of the ferrous iron, and it will be clear from the
above equation that one atom of copper is equivalent to one atom
of iron. This method gives very good results and is not difficult
to carry out as cuprous oxide dissolves quickly in acid solutions
of ferric sulphate.

Determination of nitrites

Nitrites can be oxidized quantitatively to nitrates by potassium

permanganate in presence of dilute sulphuric acid, the reaction taking place in accordance with the equation:

$$2KMnO_4 + 3H_2SO_4 + 5NaNO_2$$
$$= K_2SO_4 + 2MnSO_4 + 5NaNO_3 + 3H_2O.$$
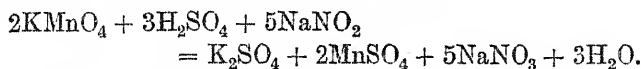
The solutions must be heated, and owing to the instability of free nitrous acid in aqueous solution, certain precautions are required. One method of procedure is to make a rough preliminary titration of the volume of potassium permanganate required to oxidize a given volume of the dilute solution of the nitrite acidified with dilute sulphuric acid, and heated to about 80° C. In a second experiment, the necessary volume of potassium permanganate is added to the measured volume of the nitrite, dilute sulphuric acid is then added, and the mixture heated to about 80° C. Potassium permanganate is then added cautiously from the burette until the reaction is completed.

Lunge has devised a better method for carrying out the determination. The solution of the nitrite is allowed to flow from the burette into a measured volume of potassium permanganate, previously acidified with dilute sulphuric acid, and heated to 40–50° C. This procedure avoids the risks due to the instability of nitrous acid, since the latter is liberated in presence of potassium permanganate, and is at once oxidized to nitric acid.

A third method for effecting quantitative oxidation is to allow the nitrite to react with a known volume of potassium permanganate in presence of dilute sulphuric acid, the permanganate being in moderate excess. The excess of permanganate is then determined iodometrically, by adding excess of potassium iodide and titrating the liberated iodine with standard thiosulphate solution as described in Chapter v.

The valuation of manganese dioxide

Manganese dioxide, MnO_2, is a substance which is very frequently contaminated with impurities. It is important, therefore, to have a reliable method of determining the percentage of MnO_2 which a sample of the commercial substance contains. One method of determining the percentage of MnO_2 in pyrolusite which is frequently employed consists in treating the substance with a measured excess of standard oxalic acid in presence of dilute

sulphuric acid when the manganese dioxide oxidizes the oxalic acid to carbon dioxide and water according to the equation

$$MnO_2 + H_2C_2O_4 + H_2SO_4 = MnSO_4 + 2H_2O + 2CO_2.$$
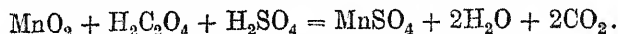
The excess of oxalic acid remaining over is then determined by titration with standard potassium permanganate in the ordinary way.

In carrying out a determination of manganese dioxide by this method, a suitable quantity, say 0·5 gramme of the sample, is weighed out and digested with a measured quantity of oxalic acid which must be in considerable excess of the amount which is theoretically required to react with the quantity of manganese dioxide taken, along with a sufficient quantity of dilute sulphuric acid. The mixture is heated to a temperature in the neighbourhood of 70° C., and the action allowed to continue until there is no black residue remaining undissolved. The presence of a white residue does not interfere as it probably consists of insoluble siliceous matter. The liquid may now be directly titrated with permanganate, but it is perhaps preferable to make up the solution to a known volume after filtration from any insoluble residue, and then to withdraw aliquot portions of the solution for titration.

It is to be noted that this procedure is simply a method of determining the amount of available oxygen in the sample, since certain other oxides of manganese are capable of decomposing oxalic acid in presence of sulphuric acid. Thus both manganese sesquioxide, Mn_2O_3, and trimanganic tetroxide, Mn_3O_4, can effect the decomposition, and if either of these oxides be present, the calculation of the percentage of the dioxide in the sample will not really indicate the true amount of this constituent.

Another method of determining the percentage of manganese dioxide in pyrolusite is described on p. 54.

Oxidations by potassium permanganate in neutral and alkaline solutions

Although by far the greater number of determinations which are carried out with potassium permanganate are performed in presence of sulphuric acid, the maximum oxidizing action being obtained under those conditions, there are certain cases more

particularly among organic compounds in which it is not possible to effect the determinations in acid solution. Some substances can however be determined in neutral or alkaline (sodium carbonate) solution. In neutral or alkaline solution potassium permanganate possesses three atoms of available oxygen, the decomposition of the molecule taking place in the manner represented by the equation

$$2KMnO_4 = K_2O + 2MnO_2 + 3O.$$
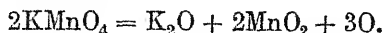
In all such cases, manganese dioxide is precipitated in a hydrated form, and the appearance of this precipitate in the liquid undergoing titration is a disadvantage, as it makes the end-point somewhat difficult to recognize.

It will be obvious, therefore, that the equivalent weight of potassium permanganate is different according as the salt is used in acid or in alkaline solution. In acid solution, two molecular proportions of the salt, 316 parts by weight, yield 80 parts by weight of available oxygen. The equivalent weight of the salt, i.e. the weight of the salt corresponding to 8 parts by weight of available oxygen, is therefore 31·6. On the other hand, when the salt is used in alkaline solution, 316 parts by weight of it yield only 48 parts by weight of available oxygen, from which it follows that the equivalent weight must be $\frac{316}{6}$ or 52·67.

Formic acid is a substance which when converted into sodium formate by the addition of excess of sodium carbonate can be determined by potassium permanganate in this way. The oxidation of formic acid takes place in accordance with the equation

$$HCOOH + O = H_2O + CO_2.$$
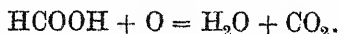
If n_1 c.c. of the solution of formic acid in presence of excess of sodium carbonate require the addition of n_2 c.c. of potassium permanganate, the weight, x, of formic acid in grammes per c.c. is determined by the equation

$$\frac{n_1 x}{n_2 w} = \frac{3 \times 46}{2 \times 158},$$

where w is the weight of potassium permanganate in grammes per c.c.

In carrying out a determination of formic acid by this method, the following procedure should be adopted. A measured quantity of the solution of the acid is placed in an evaporating dish, and made distinctly alkaline by adding a slight excess of a solution of sodium carbonate. The liquid is then heated, and the standard solution of potassium permanganate added from a burette with constant stirring until the liquid above the precipitate assumes a permanent pink colour.

Determination of manganese by potassium permanganate

If a solution of potassium permanganate be added to a nearly neutral and hot solution of a manganous salt, a precipitate of hydrated manganese dioxide is obtained by the mutual interaction of the two salts. The reaction may be represented by the equation

$$2KMnO_4 + 2H_2O + 3MnSO_4 = 5MnO_2 + 2H_2SO_4 + K_2SO_4.$$
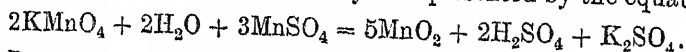
Potassium permanganate reacts here with three atoms of available oxygen. However, the reaction does not always take place in quite so simple a manner as is represented by the equation, temperature being a factor which requires to be carefully observed. Below 85° C. the reaction does take place according to the equation given, above that temperature free oxygen may be evolved leading to too great a volume of permanganate being required. It has also been found that the action is somewhat more regular in presence of certain salts, zinc salts being specially efficacious. It is perhaps scarcely necessary to point out that other oxidizable substances must be absent.

In carrying out a determination of manganese by this method, the standard permanganate is added from the burette to the hot solution of the manganous salt, the vessel being frequently shaken, until a slight excess of the reagent is indicated by the appearance of a permanent pink colour in the liquid. When the conditions are carefully observed, this method is capable of giving satisfactory results.

Note on the formula of potassium permanganate

Potassium permanganate was formerly and occasionally is still represented by the formula $K_2Mn_2O_8$. This formula clearly represents the fact that the substance possesses five atoms of

available oxygen, since the molecule may be regarded as composed of $K_2O + 2MnO + 5O$. On the other hand, although the formula may be regarded as convenient from the point of view of volumetric analysis, there is definite experimental evidence in favour of the simpler formula $KMnO_4$. Ostwald has established the empirical law that the difference between the equivalent conductivities of the sodium salts of acids at dilutions of 32 litres and 1024 litres is a function of the basicity of the acid. The difference is approximately 10 units for a monobasic acid, 20 units for a dibasic acid, 30 units for a tribasic acid and so on. Conductivity determinations have shown that permanganic acid is a monobasic acid, and consequently potassium permanganate is to be represented by the formula $KMnO_4$. It is perhaps unnecessary to point out that such evidence applies only to the molecular condition of the substance in solution; as regards the molecular weight of solid potassium permanganate we possess no definite knowledge whatever.

Determinations of the depression of freezing point in dilute solutions lead to the same conclusion. The value of van 't Hoff's coefficient i is 1·92 for solutions containing 0·01 mol of the salt per litre, from which it must follow that the solution contains only the ions $\overset{+}{K}$ and $\overset{-}{MnO_4}$.

CHAPTER III

POTASSIUM DICHROMATE AS A VOLUMETRIC OXIDIZING AGENT

Potassium dichromate $K_2Cr_2O_7$ is an orange coloured crystalline solid possessed of powerful oxidizing properties. In aqueous solution and in presence of free acid it is capable of effecting the oxidation of certain substances which are frequently determined volumetrically by potassium permanganate. Potassium dichromate, however, possesses certain undoubted advantages over permanganate. It may be used in presence either of hydrochloric or of sulphuric acid, and the aqueous solution preserves its strength unchanged for an indefinite period. Further, the solution may be used in a burette with a rubber joint. Potassium dichromate is most commonly employed for the determination of iron by oxidation from the ferrous to the ferric condition.

In presence of hydrochloric or sulphuric acid, one molecule of potassium dichromate gives rise to three atoms of available oxygen

$$4H_2SO_4 + K_2Cr_2O_7 = K_2SO_4 + Cr_2(SO_4)_3 + 4H_2O + 3O,$$
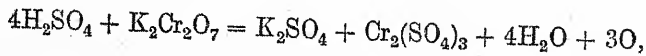
so that the conversion of ferrous chloride into ferric chloride takes place in accordance with the equation

$$K_2Cr_2O_7 + 14HCl + 6FeCl_2 = 2KCl + 2CrCl_3 + 6FeCl_3 + 7H_2O.$$
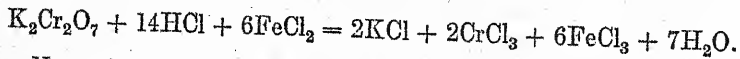
Now since one molecule of potassium dichromate possesses three atoms of available oxygen, and since three atoms of available oxygen are equivalent to six atoms of available hydrogen, the normal solution of this substance should contain one-sixth of 294·2 grammes dissolved in one litre, or approximately 49 grammes per

litre.   It is usual, however, to employ this reagent of deci-normal strength, the solution containing therefore 4·9 grammes of the salt in one litre.

Standardization of the solution

Potassium dichromate is nowadays obtainable in a state approximating to chemical purity.   There is therefore no difficulty in making up any quantity of the solution by dissolving the weighed quantity of the salt in water and diluting the resulting solution to the necessary volume.   It is necessary, however, in all cases, to standardize the solution, and this may be done very satisfactorily by means of ferrous ammonium sulphate.

When an acid solution of a ferrous salt undergoes oxidation by potassium dichromate, the orange colour of the dichromate disappears, and at the same time the solution becomes green in consequence of the presence of the chromic salt which is formed. No precise change of colour occurs at the point at which all the iron is converted into the ferric condition.   It is necessary therefore to determine the end-point of the reaction by means of an external indicator.   For this purpose potassium ferricyanide is always employed.   Ferrous salts react with the ferricyanide with formation of blue ferrous ferricyanide (Turnbull's blue), while ferric salts merely produce a brown colour with potassium ferricyanide.   In order to employ this reagent successfully, it is necessary to have the solution freshly prepared and dilute.   Drops of freshly prepared and very dilute potassium ferricyanide are placed on a white tile, and drops of the liquid undergoing titration are removed by means of a glass rod, and brought in contact with the ferricyanide.   The glass rod is of course cleaned after each test.   As long as any ferrous iron is present, the blue colour of ferrous ferricyanide makes its appearance, but when the iron is completely in the ferric condition, only a faint brown colour is formed.   The end-point can be determined with great exactness if the ferricyanide solution is prepared properly: a little practice will soon enable the operator to ascertain the strength of solution which gives the best result.

The calculation of the strength of the potassium dichromate

solution from the titration by ferrous ammonium sulphate is made
from the following equation

$$\frac{v \times \text{weight of iron per c.c.}}{v' x}$$
$$= \frac{\text{equivalent of iron}}{\text{equivalent of potassium dichromate}} = \frac{6 \times 56}{294},$$

where v denotes the volume of the iron solution taken for the
titration, v' the volume of potassium dichromate solution required
for the complete oxidation of the iron, and x the weight of potassium
dichromate in each cubic centimetre of the solution.

Determination of ferric iron by dichromate

As in the case of the determination of ferric iron by potassium
permanganate, so in the use of potassium dichromate, it is necessary
first to reduce solutions containing iron in the ferric condition to
the ferrous state. As far as the processes of reduction by hydrogen
sulphide and by sulphur dioxide are concerned, the procedure is
exactly as that already described in Chapter II. The use of
zinc and sulphuric acid for the reduction of ferric solutions which
are subsequently to be titrated by potassium dichromate is not
to be recommended, since the zinc in solution reacts with the
potassium ferricyanide employed as an indicator with formation
of zinc ferricyanide, thereby greatly obscuring the sharpness of
the end-point. There is, however, one very good method of re-
duction available for dichromate titrations, but it should not
be employed with permanganate. This consists in the use of
stannous chloride as a reducing agent, which reacts with ferric
chloride according to the equation

$$SnCl_2 + 2FeCl_3 = 2FeCl_2 + SnCl_4.$$
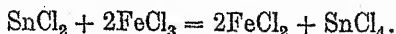
The excess of the reducing agent is removed from the solution by
precipitation with excess of mercuric chloride

$$SnCl_2 + 2HgCl_2 = 2HgCl + SnCl_4.$$
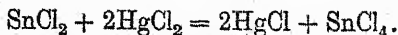
In carrying out the reduction by means of stannous chloride,
the measured quantity of the iron solution is diluted somewhat
with water, and a moderate quantity of hydrochloric acid added;

stannous chloride solution is then added cautiously to the heated iron solution until the colour vanishes.   A skilled experimenter can judge with the eye when reduction is complete, but it is certainly advisable to test in the usual way with ammonium thiocyanate that all the iron is in the ferrous condition.   After cooling, the excess of stannous chloride is removed by the addition of a slight excess of mercuric chloride.   This method of reduction when carefully carried out gives extremely satisfactory results. It is important to avoid adding too large an excess of stannous chloride to effect the reduction of the iron, as a correspondingly large excess of mercuric chloride must be added afterwards, and while the presence of a moderate amount of mercurous chloride does not interfere with the titration, precipitation of *mercury* will ruin the determination.

Determination of the reducing power of a solution of stannous chloride

A solution of stannous chloride finds application in a number of volumetric processes as a quantitative reducing agent.   Owing to its liability to undergo oxidation, a solution of stannous chloride should be preserved in an atmosphere of hydrogen, or should at least be protected from atmospheric oxygen by storing it in a bottle to which air can gain access only through a strongly alkaline solution of pyrogallol.

The reducing power of such a solution is very easily determined by allowing known volumes of the solution to react with an excess of ferric chloride solution, and then determining by titration with standard potassium dichromate the amount of ferrous iron produced.   From the equation

$$SnCl_2 + 2FeCl_3 = SnCl_4 + 2FeCl_2$$
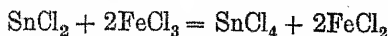
it is clear that 190 parts by weight of stannous chloride react with 2×55.85 parts by weight of metallic iron.   If the dichromate titration shows that w grammes of ferrous iron per c.c. are formed after reduction with stannous chloride, this is clearly equivalent to $\dfrac{190w}{2 \times 55.85}$ grammes of stannous chloride per c.c.   Very frequently however the reducing power of the solution is expressed in terms

of metallic iron, *e.g.* 1 c.c. of the stannous chloride is equivalent to g grammes of iron.

It is clear from what has been stated that the above process constitutes a method for the determination of tin in the stannous condition, 119 parts by weight of that metal being equivalent to 2×55.85 parts by weight of iron. The method gives very satisfactory results.

Potassium dichromate is of very great value as a volumetric reagent for the estimation of iron in ores. The procedure to be followed in order to effect the solution of the ore varies slightly with the nature of the other constituents in the ore, and a special treatise should be consulted for details, particularly as regards those ores which are not completely soluble in acids. As has been stated already, the availability of potassium dichromate in presence of hydrochloric acid, as well as the fact that its aqueous solution may be preserved of constant strength for an indefinite length of time, makes it preferable to potassium permanganate for many purposes. On the other hand it possesses the disadvantage of requiring an external indicator, which makes the titrations take a longer time.

Determination of oxidizing agents

Substances which oxidize ferrous salts quantitatively to the ferric condition can be determined by a method similar to that already described in connexion with potassium permanganate, viz., the addition of a known excess of the ferrous salt to the oxidizing agent, and subsequent titration by potassium dichromate of the amount of ferrous salt remaining in excess. Chlorates may be determined in this way, reduction of the ferrous salt taking place thus,

$$KClO_3 + 6FeSO_4 + 3H_2SO_4 = KCl + 3Fe_2(SO_4)_3 + 3H_2O.$$

Acid solutions of thallic salts may be determined in a similar manner, reduction from the tervalent thallic to the univalent thallous condition proceeding as follows:

$$Tl_2O_3 + 4FeO = Tl_2O + 2Fe_2O_3.$$
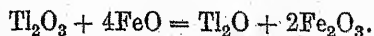
The excess of ferrous sulphate is then titrated by potassium dichromate. In this case the use of potassium permanganate is

inadmissible because thallous salts in acid solution are oxidized by permanganate, but are stable towards dichromate (Berry, *Trans. Chem. Soc.*, 1922, **121**, 394, and 1923, **123**, 1109).

Standardization of potassium dichromate by means of iron wire

Iron wire was formerly, and still is, largely used to determine the exact strengths of solutions of potassium dichromate and also of potassium permanganate; a solution of ferrous sulphate being prepared by dissolving a known weight of iron wire in dilute sulphuric acid, and making the solution up to some definite volume. It is not difficult to obtain iron wire containing 99·6 per cent. of pure iron, and for most purposes this degree of purity is quite sufficient; but if the strength of the volumetric oxidizing agent is required to be known with great exactness, pure iron prepared by the electrolysis of ferrous oxalate should be employed. When iron of the order of 99·6 per cent. purity is employed, some operators multiply the weight of iron taken by 0·996 in order to correct for the small amount of impurity present, but it is very doubtful if the correction is a justifiable one, as it has been found that some of the impurities have a reducing action on permanganate.

In effecting the solution of iron wire, it was formerly the custom to take somewhat elaborate precautions to prevent the access of air to the solution in order to avoid oxidation of the solution; but it has gradually become recognized that solutions of ferrous salts are much more stable towards atmospheric oxygen than is commonly supposed. Solutions of ferrous sulphate are certainly much more stable than solutions of ferrous chloride; it is advisable therefore to use sulphuric acid in preference to hydrochloric for dissolving the metal. For most ordinary work, the solution of the metal may be effected very conveniently in a flask which is heated on a hot plate, a small funnel being placed in the neck of the flask during the operation. The contents are then washed carefully into a standard flask and diluted to a known volume. Aliquot portions are then taken for titration by potassium dichromate.

CHAPTER IV

DETERMINATIONS WITH STANDARD IODINE

A solution of iodine in potassium iodide finds frequent application in analysis as a volumetric oxidizing agent. Thus it is capable of converting arsenious and antimonious compounds into the corresponding arsenic and antimonic compounds. If employed in conjunction with a standard solution of sodium thiosulphate, its range of application is capable of being extended considerably. We shall, however, in the present chapter restrict our discussion to the determination of certain reducing agents by standard iodine alone.

Preparation of a deci-normal solution of iodine

Since iodine is a monovalent element and since its atomic weight is 126·92, a normal solution will contain that weight of the element in grammes dissolved in one litre. In practice one never employs a solution of greater strength than deci-normal; the necessary quantity of iodine in a solution of deci-normal strength will therefore be 12·692 grammes per litre or for approximate purposes 12·7 grammes per litre.

Commercial resublimed iodine may contain various impurities including cyanogen iodide. It may be purified to a considerable extent by mixing it with potassium iodide and subliming. It is, however, in general of no advantage to take elaborate precautions to prepare iodine of a high degree of purity; it is better to prepare a solution of approximately deci-normal strength, and then to determine its exact strength in one of the ways about to be described. In weighing iodine, it is to be observed that the

volatility of this element may make an exact weighing a difficult matter, unless the substance be weighed in a closed vessel.

The weighed quantity of iodine is dissolved in a solution of potassium iodide. The weight of potassium iodide to be taken should be from one-and-one-half times to twice that of the iodine. The solution is then diluted with distilled water to the necessary volume. Heat should not be employed to accelerate the dissolution of the iodine on account of the volatility of the element. If the solution be preserved in dark well-stoppered bottles, it will retain its strength unaltered for a long period. This reagent must always be employed in a burette fitted with a glass tap, as it makes rubber tubing useless.

An excellent method of standardizing a solution of iodine is by the use of arsenious oxide purified by resublimation. A small quantity of arsenious oxide is heated in a dish covered with another vessel. The resublimed arsenious oxide should be perfectly white in colour. About 5 grammes of the solid are weighed out accurately, dissolved in an aqueous solution containing about four times the weight of pure sodium carbonate, and then diluted to one litre. In this way a standard solution of arsenious oxide in the form of sodium arsenite is obtained.

When iodine is added to a solution of an alkaline arsenite, oxidation of the arsenic takes place in accordance with the equation

$$As_2O_3 + 2I_2 + 2H_2O \rightleftharpoons As_2O_5 + 4HI,$$
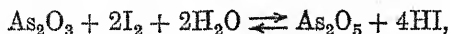
two molecules of iodine (four atoms) oxidizing one molecule of arsenious to arsenic oxide. It will be observed, from the above equation, that free hydrogen iodide is formed in the reaction. Now hydrogen iodide is a reducing reagent, and the reaction represented above is reversible. Consequently it is necessary to add some substance which will combine with the hydriodic acid to enable the oxidation of the arsenious oxide to proceed to completion. For this purpose it is usual to employ sodium bicarbonate $NaHCO_3$. Caustic alkalis must not be employed, since they react with iodine with formation of iodide and hypoiodite. A moderate excess of a solution of sodium bicarbonate should therefore be added in each titration.

The end-point is observed with great precision by the addition of a small quantity of a solution of starch to the liquid undergoing titration. The smallest quantity of free iodine in excess will then be recognized by the appearance of the well-known blue colour which this element forms with starch. The preparation of the starch solution is a matter of importance. The best plan is to add to about 100 c.c. of boiling water a small quantity of starch ground up with a little water, and to continue the boiling for a few minutes. It is important to avoid taking too much starch; if too much starch be used the resulting solution will become gelatinous. It is advisable though not necessary to filter the starch solution before using it as an indicator. When carefully prepared, the most minute quantity of free iodine dissolved in potassium iodide can be detected by the appearance of a dark blue colour.

If x be the weight of iodine in grammes in each cubic centimetre of the solution, and if n_1 c.c. of the iodine solution require n_2 c.c. of alkaline arsenite solution containing w grammes of As_2O_3 per c.c., the strength of the iodine solution is determined by the equation

$$\frac{n_1 x}{n_2 w} = \frac{\text{equivalent of iodine}}{\text{equivalent of arsenious oxide}} = \frac{127 \times 4}{198}.$$

Other methods of standardizing iodine

A solution of iodine may be standardized in various other ways than that already described. If a standard solution of sodium thiosulphate is available, the strength of an iodine solution may be determined with accuracy by titrating the one solution against the other, when interaction takes place with formation of sodium iodide and tetrathionate, according to the equation

$$I_2 + 2Na_2S_2O_3 = Na_2S_4O_6 + 2NaI.$$
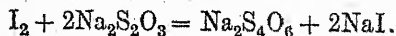
If the exact strength of the thiosulphate solution is not known, but the experimenter be provided with a standard solution of potassium permanganate or dichromate, the strength of the thiosulphate solution may be determined by allowing the solution of potassium permanganate or dichromate acidified with sulphuric

acid to react with an excess of potassium iodide, when iodine is liberated quantitatively in accordance with the equations

$$2KMnO_4 + 8H_2SO_4 + 10KI = 6K_2SO_4 + 2MnSO_4 + 8H_2O + 5I_2$$

and

$$K_2Cr_2O_7 + 7H_2SO_4 + 6KI = 4K_2SO_4 + Cr_2(SO_4)_3 + 7H_2O + 3I_2,$$
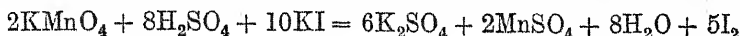
in the cases of the permanganate and dichromate respectively. The liberated iodine is then titrated with sodium thiosulphate solution, the end-point being determined with starch, and then the iodine solution, the strength of which is required, is determined by titration against the solution of sodium thiosulphate. The experimental procedure in connexion with the determination of iodine by sodium thiosulphate will be described in greater detail in the next chapter.

Determination of antimony by standard iodine

Antimony in the antimonious condition may be determined with accuracy by titration with iodine, the element undergoing oxidation from the antimonious to the antimonic condition according to the equation

$$Sb_2O_3 + 2I_2 + 2H_2O \rightleftarrows Sb_2O_5 + 4HI.$$
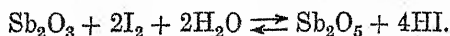
As in the case of the reaction between arsenious oxide and iodine, so in the present case, the reaction is reversible owing to the reducing action of the hydrogen iodide on the antimonic oxide. For this reason it is as before necessary to suppress the reverse reaction by the addition of a sufficient excess of sodium bicarbonate. It is necessary also to add a sufficient quantity of tartaric acid or of Rochelle salt (potassium sodium tartrate $KNaC_4H_4O_6$) to prevent the precipitation of basic salts of the metal as the result of hydrolysis.

If the metal is present in the antimonic condition to start with, it is necessary to reduce it to the antimonious condition before titration with standard iodine. Sulphur dioxide is an excellent reducing agent for this purpose. The reduction is carried out in presence of hydrochloric acid, and the excess of sulphur dioxide removed by prolonged boiling. The solution is

then made just alkaline by caustic soda, and a slight excess of tartaric acid added. After addition of excess of sodium bicarbonate, the solution is titrated with standard iodine in the usual manner, using starch as indicator.

Determination of tin

A solution of iodine is capable of oxidizing tin from the stannous condition to the stannic condition quantitatively, one molecule (two atoms) of iodine effecting the conversion of one atom of tin from the stannous to the stannic condition. The determination can be carried out directly in presence of hydrochloric acid. Acid solutions of stannous chloride are less sensitive as regards oxidation than alkaline ones, and consequently the method is preferable to that of working in alkaline solution in which the stannous salt is treated with sodium bicarbonate and potassium sodium tartrate before titrating with iodine. The end-point is obtained in the usual manner by the use of starch. This method of determining tin gives results of very fair accuracy; the chief precaution to be observed is to prevent the absorption of oxygen from the air by the stannous salt.

Determination of sulphur dioxide in aqueous solution

An approximate determination of sulphur dioxide in solution may be made by allowing the aqueous solution of the gas to react with iodine in accordance with the equation

$$I_2 + SO_2 + 2H_2O = H_2SO_4 + 2HI.$$
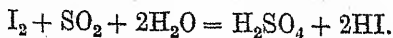
It was shown by Bunsen that the reaction only takes place in accordance with the above equation when the solution of sulphur dioxide is fairly dilute (not greater than 0.04 per cent. of SO_2 by weight). The irregularities which occur with more concentrated solutions appear to be due, in part at any rate, to the fact that under certain conditions the hydrogen iodide reduces some of the sulphur dioxide to free sulphur. It has subsequently been found that oxidation of the sulphur dioxide to sulphuric acid takes place quantitatively in the manner indicated by the above equation if the solution of sulphur dioxide is run into the solution of iodine,

not *vice versa*, even when the solution of sulphur dioxide is fairly concentrated. Consequently it is not advisable to attempt the direct titration of this substance by means of standard iodine; it is much better to add the solution of sulphur dioxide to a known excess of iodine and then to determine the excess by means of sodium thiosulphate. It will be seen from the above equation that 127 parts by weight of iodine react with 32 parts by weight of sulphur dioxide.

Determination of sulphuretted hydrogen in aqueous solution

Iodine and sulphuretted hydrogen interact with formation of hydrogen iodide and sulphur in accordance with the equation

$$I_2 + H_2S = 2HI + S.$$
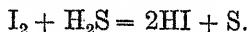
For various reasons the direct titration of aqueous solutions of this gas by means of iodine does not give accurate results. It is stated that greater accuracy may be attained by adding the solution of hydrogen sulphide to a measured excess of iodine, and subsequently determining the excess by sodium thiosulphate as in the case of sulphur dioxide already described.

A method of procedure which yields satisfactory results is to allow the solution of sulphuretted hydrogen to react with excess of a standard solution of arsenious oxide in presence of hydrochloric acid, when arsenious sulphide is precipitated in accordance with the equation

$$As_2O_3 + 3H_2S = As_2S_3 + 3H_2O.$$
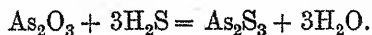
The precipitated arsenious sulphide is separated by filtration, and the filtrate and washings titrated by means of standard iodine in the usual manner, starch being employed as indicator. Since three molecules of hydrogen sulphide react with one molecule of arsenious oxide, it is clear that 3×34 parts by weight of sulphuretted hydrogen are equivalent to 198 parts by weight of arsenious oxide. In carrying out the determinations, the best plan is to allow the solution of the gas to flow into a measured excess of standard alkaline arsenite solution. Hydrochloric acid is then added in sufficient excess to react distinctly acid to indicators.

The presence of free acid is necessary to precipitate the arsenic, otherwise a colloidal solution of arsenious sulphide will be obtained. The filtrate and washings are then made alkaline by the addition of excess of sodium bicarbonate, and the determination of the excess of arsenious oxide effected by titration with standard iodine.

The reaction between hydriodic and iodic acids

In the absence of free acid, iodides and iodates are stable when present together in aqueous solution, but if an acid is added, liberation of iodine takes place at once, thus

$$KIO_3 + 5KI + 6HCl = 6KCl + 3H_2O + 3I_2.$$
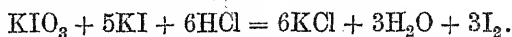
The iodine which is liberated remains in solution if excess of potassium iodide is present. As potassium iodate and potassium iodide are both salts which are readily obtainable in a high degree of purity, this reaction affords a convenient means of obtaining a standard solution of iodine, and is well adapted for standardizing solutions of sodium thiosulphate.

From the equation, it is clear that the equivalent weight of potassium iodate in this reaction is one-sixth of its formula weight, i.e. one-sixth of 214, or approximately 35·7. A deci-normal solution of potassium iodate will contain therefore 3·57 grammes of the salt per litre.

CHAPTER V

## THE DETERMINATION OF IODINE BY STANDARD SODIUM THIOSULPHATE

In the previous chapter we had occasion to refer to the reaction between iodine and sodium thiosulphate resulting in the formation of sodium iodide and tetrathionate. The importance of this reaction is that it affords a means of determining not only free iodine, but all substances which liberate iodine quantitatively from potassium iodide on acidifying; in other words a large number of oxidizing agents may be determined by standard sodium thiosulphate. The reaction between iodine and sodium thiosulphate is one of the most sensitive and accurate in the whole domain of volumetric analysis, and its range of application is very great.

Sodium thiosulphate crystallizes with five molecules of water, but there seems to be a slight doubt regarding the constancy of the water of crystallization of this substance. The exact strength of the solution should therefore be determined in one of the ways about to be described.

Standardization of an approximately deci-normal solution

An approximately deci-normal solution may be prepared by dissolving one-tenth of the gramme molecular weight of sodium thiosulphate pentahydrate in water and diluting the solution to one litre. If one is in possession of a standard solution of iodine, the determination of the strength of the thiosulphate solution is effected with great ease, the end-point being determined in the usual manner with starch.

From the equation

$$I_2 + 2Na_2S_2O_3 = Na_2S_4O_6 + 2NaI$$
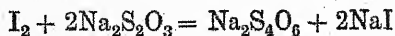
it is clear that 127 parts by weight of iodine react with 158 parts by weight of sodium thiosulphate calculated as anhydrous salt.

The solution of sodium thiosulphate may be standardized in other ways with equally good results. In connexion with the standardization of iodine, it was mentioned that a solution of thiosulphate might be determined with reference to a standard solution of potassium permanganate or dichromate. We shall now describe in somewhat greater detail the procedure to be followed in determining the exact strength of a thiosulphate solution by one or other of these oxidizing agents.

A given volume (say 20 c.c.) of a standard solution of potassium permanganate is measured out, acidified with dilute sulphuric acid and diluted somewhat with water. A few crystals of potassium iodide are now added and the liquid stirred. Iodine is liberated quantitatively in accordance with the equation

$$2KMnO_4 + 8H_2SO_4 + 10KI = 6K_2SO_4 + 2MnSO_4 + 8H_2O + 5I_2.$$
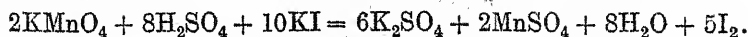
Sodium thiosulphate is then added from a burette when the liberated iodine dissolved in the excess of potassium iodide is gradually removed with formation of sodium iodide and tetra-thionate. When only a faint yellow colour of iodine remains, starch solution is added, and the addition of sodium thiosulphate continued until the deep blue liquid becomes perfectly colourless. The end-point is determined with great precision; a single drop of sodium thiosulphate should be sufficient to cause a striking disappearance of colour.

Since according to the preceding equation two molecules of potassium permanganate liberate five molecules of iodine, and since one molecule of iodine reacts with two molecules of sodium thiosulphate, it is clear that two molecules of potassium permanganate are equivalent to ten molecules of sodium thiosulphate. If therefore n_1 c.c. of potassium permanganate containing w grammes of the salt per c.c. require n_2 c.c. of sodium thiosulphate, the weight of sodium thiosulphate x in grammes per cubic centimetre is given by the equation

$$\frac{n_2 x}{n_1 w} = \frac{10 \times 158}{2 \times 158}.$$

If potassium dichromate is employed for standardizing the

thiosulphate solution, the procedure to be followed is very similar to that above described. In determining the end-point, it is important to remember that a green chromic salt is formed by the reduction of the dichromate; the completion of the reaction is therefore that point at which the solution changes from the deep blue of starch iodide to pale green. As has been explained in the previous chapter, the liberation of iodine from potassium iodide by means of acidified potassium dichromate takes place in accordance with the equation

$$K_2Cr_2O_7 + 7H_2SO_4 + 6KI = 4K_2SO_4 + Cr_2(SO_4)_3 + 7H_2O + 3I_2.$$

The reaction is however considerably slower than when potassium permanganate is used. Consequently if n_1 c.c. of potassium dichromate, containing w grammes of the substance dissolved in each cubic centimetre of the solution, require n_2 c.c. of sodium thiosulphate of which the weight of salt x in grammes per cubic centimetre is required, x is found by the solution of the equation

$$\frac{n_2 x}{n_1 w} = \frac{6 \times 158}{294}.$$

Determination of oxidizing agents by potassium iodide and sodium thiosulphate

The examples already given of the use of standard potassium permanganate or dichromate for the determination of the exact strength of a solution of sodium thiosulphate may clearly be applied in the reciprocal way. That is to say, if one is in possession of a standard solution of sodium thiosulphate, one may determine with accuracy the strength of a solution of a dichromate or of a permanganate. The number of substances which may be determined by such a procedure is very great, and we shall discuss some individual examples in the course of the present chapter. There is, however, one general remark which must be made before proceeding further. The velocity of the reaction between potassium iodide and some oxidizing agents in acid solution is frequently not great at ordinary temperatures, that is to say, the liberation of iodine takes place slowly in many cases, particularly towards the end of the reaction, when the mass concentration of

the oxidizing agent has fallen to a small value.   Consequently in titrating the liberated iodine by means of sodium thiosulphate, the blue colour of starch iodide frequently makes its appearance after the titration is apparently completed.   More sodium thiosulphate must then be added until the liberation of iodine no longer takes place.

Determination of hydrogen peroxide

This substance may be determined iodometrically with great accuracy.   The measured quantity of the solution of the peroxide is acidified with dilute sulphuric acid, and excess of potassium iodide added.   Iodine is liberated quantitatively in accordance with the equation

$$2KI + H_2SO_4 + H_2O_2 = 2H_2O + K_2SO_4 + I_2.$$
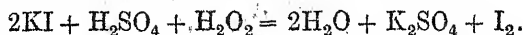
The liberated iodine is then determined by means of standard sodium thiosulphate in the usual manner;   one molecule of hydrogen peroxide being equivalent to two molecules of sodium thiosulphate.

The chemical dynamics of the reaction between hydriodic acid and hydrogen peroxide has been made the subject of an exhaustive investigation by Harcourt and Esson.   These investigators showed that when the active mass of the hydriodic acid was kept approximately constant by the addition of known constant amounts of sodium thiosulphate as soon as free iodine made its appearance, the rate of disappearance of the hydrogen peroxide was at every instant proportional to the amount present, or

$$-\frac{dC}{dt} = kC.$$

It is clear from this equation that theoretically the whole of the hydrogen peroxide will only be decomposed after an infinite time, and as a matter of fact inaccurate results are frequently to be traced to stopping the titration too soon.

In determining hydrogen peroxide by this method, it is important to add a considerable excess of sulphuric acid.   The necessity of a large excess does not appear to be very obvious, but there appears to be little doubt that the inaccurate results

obtained by some chemists are due to the use of too little acid. A trace of ammonium molybdate should be added to catalyse the reaction. The permanganate method of determining hydrogen peroxide described in Chapter II is liable to give high results, as the organic preservatives which are usually present are themselves oxidized under these conditions. No such objection can be raised against the iodometric method, as special experiments have shown that it yields perfectly accurate results in presence of any of the usual preservatives such as glycerol.

Determination of copper

An acidified solution of a cupric salt will liberate iodine quantitatively from potassium iodide with formation of an equivalent amount of cuprous iodide at the same time. Upon this reaction an accurate method of determining copper has been based. It has been shown that the reaction is somewhat irregular in presence of certain mineral acids, but that in presence of acetic acid the following reaction takes place:

$$2Cu(C_2H_3O_2)_2 + 4KI = Cu_2I_2 + 4C_2H_3O_2K + I_2.$$
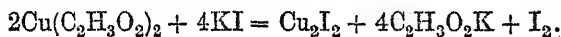
The free iodine is determined by titration with standard sodium thiosulphate.

If the solution of the cupric salt contains any mineral acid, it is necessary to neutralize the excess of free acid by the addition of sodium carbonate in excess, and to continue the addition of the carbonate till a slight precipitate of basic cupric carbonate is obtained. The solution is then acidified by the cautious addition of acetic acid, a large excess of acetic acid being carefully avoided. A few crystals of potassium iodide are then added, when cuprous iodide is precipitated and an equivalent quantity of free iodine is set free. The liberated iodine is titrated by means of sodium thiosulphate, care being taken that the reaction is completed.

When ordinary precautions are taken, this method of determining copper gives accurate results. It is clear from the above equation that two atoms of copper (metal) liberate one molecule of iodine, which in its turn reacts with two molecules of sodium thiosulphate. The weight of copper (metal) x in each cubic

centimetre of the solution is therefore found by solving the equation

$$\frac{n_1 x}{n_2 w} = \frac{63 \cdot 6}{158},$$

where n_1 and n_2 denote the volumes of the copper solution and of the sodium thiosulphate respectively taken for the titration.

Determination of the available chlorine in bleaching powder

The precise nature of bleaching powder is still enveloped in mystery despite the large number of investigations which have been made on this substance. As is well known, when slaked lime is allowed to absorb chlorine, a product is obtained which is sometimes represented by the formula $CaOCl_2$, although it is very doubtful if there is any justification for assigning a formula to the substance at all. Some of the chlorine behaves as if present in the form of hypochlorite, and it is upon the amount of chlorine present in this form, or available chlorine as it is technically termed, that the bleaching value of a specimen of bleaching powder depends.

Bunsen has devised an excellent method of determining the percentage of available chlorine in bleaching powder by allowing the substance, acidified by acetic acid, to liberate iodine from excess of potassium iodide. The liberated iodine is then determined by titration by means of standard sodium thiosulphate. Since one molecule of chlorine liberates one molecule of iodine, it is clear that 35·5 parts by weight of chlorine are equivalent to 158 parts by weight of sodium thiosulphate.

In carrying out an estimation of the available chlorine in bleaching powder, a suitable quantity of the sample, say from 10 to 20 grammes, is weighed out, and triturated with a little water in a mortar; the milky liquid is then gradually transferred to a measuring flask and diluted to the necessary volume. The contents of the flask are well shaken, and a measured quantity of the turbid solution withdrawn by means of a pipette. A few crystals of potassium iodide are now added, the liquid diluted with a little water, and acidified with acetic acid. The liberated iodine is then determined by means of sodium thiosulphate, and the percentage of available chlorine in the sample is easily calculated.

The reason for acidifying the solution by means of acetic acid instead of hydrochloric acid is that bleaching powder frequently contains calcium chlorate, with the result that chlorine would be liberated by interaction with the hydrochloric acid added, and high results obtained.

Determination of ferric iron iodometrically

An acidified solution of a ferric salt liberates iodine from potassium iodide with formation of ferrous salt in accordance with the equation
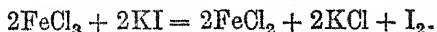
$$2FeCl_3 + 2KI = 2FeCl_2 + 2KCl + I_2.$$

The liberated iodine is then determined by titration with standard sodium thiosulphate. It is perhaps unnecessary to add that the iron solution must contain no nitric acid or other oxidizing agent. The solution is acidified by hydrochloric acid. The liberation of iodine takes place slowly, but the reaction can be catalysed by adding cuprous iodide. The potassium iodide solution is treated with a little copper sulphate and starch, and the blue colour then exactly discharged by adding sodium thiosulphate. The mixture is then added to the solution of the ferric salt, and the liberated iodine at once titrated with sodium thiosulphate.

Determination of other oxidizing agents by means of potassium iodide and thiosulphate

In general it may be stated that any substance which will liberate iodine from potassium iodide quantitatively on acidifying may be determined by the iodometric method, and the few examples which have been given will serve to indicate the wide range of this method. But the possibilities of this method have been by no means exhausted; ferricyanides, for example, may be determined with satisfactory results. It does not follow, however, that the iodometric method is necessarily the best method to employ when other methods are available; in particular the slowness of the liberation of iodine towards the end of the reaction in certain cases is an undoubted disadvantage. Some substances cannot be determined directly by this method, but may be determined by taking advantage of the fact that when heated with concentrated hydrochloric acid they evolve chlorine; the chlorine

may be allowed to liberate iodine from potassium iodide and this liberated iodine determined by sodium thiosulphate.

Determination of oxidizing agents by distillation with hydrochloric acid

Insoluble substances such as manganese dioxide may be determined with accuracy by distillation with excess of concentrated hydrochloric acid, when chlorine is liberated quantitatively. In the case of manganese dioxide, the reaction takes place in accordance with the equation

$$MnO_2 + 4HCl = MnCl_2 + 2H_2O + Cl_2.$$
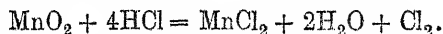
The chlorine is then passed into an excess of potassium iodide solution, when one molecule of chlorine liberates one molecule of iodine. Since one molecule of iodine reacts with two molecules of sodium thiosulphate, it is clear that one molecule of manganese dioxide is equivalent to two molecules of sodium thiosulphate.

Various forms of apparatus have been devised for carrying out distillation determinations of this kind. If ordinary corks are
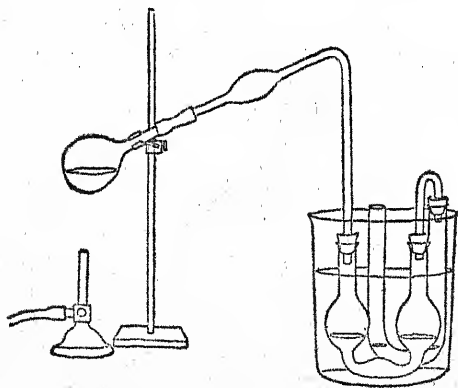

Fig. 3.

employed, they should be soaked in melted paraffin wax before use in order to protect them from the corrosive action of the chlorine, but for the most accurate work it is best to have apparatus constructed with ground glass joints.

For practice in this method a good exercise is the determination of MnO_2 in a specimen of commercial pyrolusite. About

0·5 gramme of the substance is weighed out and introduced into the distillation apparatus, a moderate excess of concentrated hydrochloric acid being added. In order to prevent regurgitation in the event of a fall of the pressure, it is advisable to add a small lump of magnesite to the contents of the distillation flask. This substance dissolves but slowly, generating a steady stream of carbon dioxide, which not only prevents regurgitation but also dilutes the chlorine and renders the absorption of the gas by the potassium iodide less violent. The absorption bulbs should be kept cool by being immersed in water during the experiment. After the reaction is completed, the contents of the absorption tubes are washed out with a little potassium iodide solution, diluted with water to some definite volume; aliquot portions are titrated by means of standard sodium thiosulphate solution, starch being added towards the end of the reaction. From the above equation the percentage of manganese dioxide in the weight of pyrolusite taken may be readily calculated.

The distillation method is clearly applicable to all substances which evolve chlorine when heated with concentrated hydrochloric acid. In general, however, its use is restricted to substances which are insoluble in water, such as lead dioxide and manganese dioxide, since soluble oxidizing agents liberate iodine from potassium iodide directly on the addition of acids.

Use of sodium thiosulphate for residual titration with iodine

In many determinations by means of iodine it is convenient not to determine the end-point of the reaction between the substance which is undergoing oxidation and iodine directly, but to add a measured quantity of standard iodine solution that is known to be in excess of the quantity required, and then to determine the excess of iodine that has been added by residual titration with standard sodium thiosulphate. For example it was pointed out in Chapter IV that the direct titration of sulphur dioxide in aqueous solution by means of iodine was not to be recommended on account of irregularities which are not encountered when the reaction is permitted to take place by allowing the solution of sulphur dioxide to flow into excess of iodine solution, the excess of iodine being determined by sodium thiosulphate.

CHAPTER VI

THE DETERMINATION OF HALIDES BY STANDARD SILVER NITRATE

General

A solution of silver nitrate finds frequent application in volumetric analysis for the determination of soluble halides by precipitation of insoluble silver halide. Soluble chlorides, bromides, and iodides are determined quantitatively by precipitation as the insoluble silver halide. Thus when a solution of silver nitrate is added to a solution of potassium chloride, double decomposition takes place in accordance with the equation

$$AgNO_3 + KCl = AgCl + KNO_3.$$
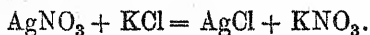
The end-point may be determined with great accuracy by taking advantage of the property which the silver halides possess of coagulating when agitated; it is thus possible to ascertain when precipitation is complete by adding the reagent very cautiously and observing whether any further precipitation takes place.

A more rapid method of determining the end-point is to add a very small quantity of a solution of potassium chromate to the liquid undergoing titration. As long as precipitation of the halide is incomplete, the liquid remains of a pale yellow colour, but as soon as the slightest excess of the silver solution has been added, the dark red colour of silver chromate imparts a characteristic pink tint to the liquid. In order to employ potassium chromate successfully as an indicator, it is essential that the solution which is undergoing titration should be exactly neutral. In presence of free acid the precipitation of silver chromate is interfered with, while free alkali causes the separation of silver oxide. For most

purposes the potassium chromate indicator must now be regarded as obsolete. Greater accuracy may be obtained by the use of adsorption indicators such as fluorescein, eosin, phenosafranine, or tartrazine as described in Chapter XIV. Moreover potassium chromate cannot be used for the titration of iodides, on account of the solubility relationships.

### Preparation of a deci-normal solution

Silver nitrate may be obtained of a high degree of purity by recrystallization. The molecular weight of this salt is 169·89, International atomic weights being used; consequently a deci-normal solution will contain 16·989 grammes or, with sufficient approximation, 17 grammes of the salt dissolved in one litre. A solution of silver nitrate prepared by weighing out the necessary quantity of the salt and diluting it to one litre may be relied upon as being of sufficient exactness for all ordinary purposes, but if the experimenter be in any doubt regarding the purity of the silver nitrate used the solution should be standardized by means of pure sodium chloride.

Instead of making up a standard solution of silver nitrate from the recrystallized salt, the solution may be prepared by starting with pure metallic silver. The weighed quantity of the metal is dissolved in pure dilute nitric acid with the aid of a gentle heat, care being taken to avoid loss of liquid by effervescence. When solution is complete the excess of acid is removed by evaporating the solution to dryness, and the residue is then dissolved in water and made up to the necessary volume.

Determination of halides

As has been explained already, all soluble chlorides, bromides, and iodides may be determined by precipitation by means of standard silver nitrate. If the solution to be titrated contains free acid, and if it be desired to employ potassium chromate as an indicator, it is necessary to neutralize the free acid before titration. In many cases the simplest method of securing neutrality is to add a slight excess of calcium carbonate to the liquid. For example hydrochloric acid may be determined by

addition of excess of calcium carbonate, when the hydrochloric acid is converted into an equivalent amount of calcium chloride which possesses a neutral reaction, and which is precipitated quantitatively by silver nitrate

$$2HCl + CaCO_3 = CaCl_2 + H_2O + CO_2,$$
$$CaCl_2 + 2AgNO_3 = Ca(NO_3)_2 + 2AgCl.$$
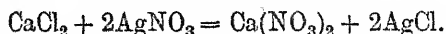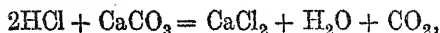
If n_1 c.c. of the hydrochloric acid solution require n_2 c.c. of silver nitrate for complete precipitation, the weight x of hydrochloric acid per cubic centimetre is obtained by the solution of the equation

$$\frac{n_1 x}{n_2 w} = \frac{\text{equivalent of hydrochloric acid}}{\text{equivalent of silver nitrate}} = \frac{36 \cdot 5}{170},$$

where w is the weight of silver nitrate dissolved in each cubic centimetre of the standard solution.

Indirect determinations by standard silver nitrate

Not only may chlorides be determined by precipitation with silver nitrate solution, but all substances which can be converted quantitatively into neutral chlorides may be determined in this way. For example the alkali salts of many organic acids are converted on ignition into carbonates; these carbonates may be decomposed by excess of dilute hydrochloric acid, and the excess of acid removed by evaporating the resulting solution to dryness. After extracting with water, the solution of sodium or potassium chloride is determined by titration with standard silver nitrate in the ordinary manner, using potassium chromate as indicator. Instead of converting the carbonates into chlorides however, it is more usual to determine the carbonates by titration with standard mineral acid, methyl orange being employed as an indicator.

Determination of two halides in a mixture

A very important application of silver nitrate as a volumetric reagent consists in the determination of two halides such as sodium chloride and potassium chloride or potassium chloride and potassium bromide when present together in solution and the total weight of the mixed salts in a given volume of solution is

known. The method depends upon the difference between the equivalent weights of the two halides, and the greater this difference, the more accurate will be the determination. A single determination with standard silver nitrate is sufficient for the estimation of the amounts of the two constituents of the mixture, as the following example will show.

If we have a mixture of sodium chloride and potassium chloride, the solution containing w_1 grammes of the mixed salts dissolved in one litre, and we find from the results of the titrations that w_2 grammes of silver nitrate are required for complete precipitation per litre, the weights of the two chlorides are obtained by solving the simultaneous equations

$$x + y = w_1 \quad \dots\dots\dots\dots\dots\dots(1),$$
$$\frac{170x}{58\cdot5} + \frac{170y}{74\cdot6} = w_2 \quad \dots\dots\dots\dots\dots(2),$$

where x and y denote respectively the weights of sodium chloride and potassium chloride in one litre of the solution.

Again if it is required to determine the weights of potassium chloride and potassium bromide in a solution containing w_3 grammes of the mixed salts dissolved in one litre, aliquot portions of the solution are titrated by means of standard silver nitrate in the usual manner, and if it be found that w_4 grammes of silver nitrate are required for complete precipitation per litre, x the weight of potassium chloride and y the weight of potassium bromide are calculated from the equations

$$x + y = w_3 \quad \dots\dots\dots\dots\dots\dots(3),$$
$$\frac{170x}{74\cdot6} + \frac{170y}{119\cdot1} = w_4 \dots\dots\dots\dots\dots(4).$$

It is clear that since the method depends upon the difference between the equivalent weights of the two constituents of the mixture, the accuracy of the process is somewhat limited. The method is most satisfactory when there is not a great difference in the relative amounts of the two constituents of the mixture; it is easy to understand that if a solution containing a relatively large amount of one constituent as compared with the other be titrated with silver nitrate, a very small error in the titration will give rise to a very considerable error in the calculated values.

Theory of errors involved in indirect analysis

Let us consider, as in the special example already discussed, a mixture of two constituents: w_1 grammes of the mixture containing x grammes of a constituent of equivalent weight m_1 and y grammes of a constituent of equivalent weight m_2. Let n be the equivalent weight of the substance employed in solution for the determination, and let w_2 grammes of this substance be required for reaction with w_1 grammes of the mixture; consequently we may write the equations

$$x + y = w_1 \quad \dots\dots\dots\dots\dots (1),$$

and
$$\frac{nx}{m_1} + \frac{ny}{m_2} = w_2 \quad \dots\dots\dots\dots\dots (2),$$

$$y = w_1 - x;$$

$$\therefore \frac{nx}{m_1} + \frac{nw_1}{m_2} - \frac{nx}{m_2} = w_2,$$

$$\therefore x\left(\frac{n}{m_1} - \frac{n}{m_2}\right) = w_2 - \frac{nw_1}{m_2},$$

$$x = \frac{w_2 - \dfrac{nw_1}{m_2}}{\dfrac{n}{m_1} - \dfrac{n}{m_2}}.$$

Differentiating with respect to w_2,

$$\frac{dx}{dw_2} = \frac{1}{n\left(\dfrac{1}{m_1} - \dfrac{1}{m_2}\right)}$$

This equation shows that the error in x due to unit error in w_2 is inversely proportional to the difference in the reciprocals of m_1 and m_2.

Determination of chlorates

All chlorates are decomposed on ignition into chlorides with evolution of oxygen. For example potassium chlorate decomposes according to the equation

$$2KClO_3 = 2KCl + 3O_2.$$
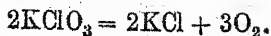
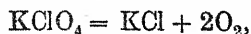As a matter of fact the decomposition is not quite so simple as that represented by this equation, since potassium chlorate on heating is converted partly into potassium perchlorate

$$4KClO_3 = 3KClO_4 + KCl.$$

But since the potassium perchlorate on heating is decomposed completely into potassium chloride with evolution of oxygen

$$KClO_4 = KCl + 2O_2,$$

the decomposition of the chlorate may be represented as taking place in accordance with the first equation.

The weighed quantity of the chlorate is ignited in a crucible until constant in weight; the residue is then extracted with water and diluted to some definite volume. Aliquot portions of the solution are then determined by titration with standard silver nitrate.

Determination of alkali cyanides in aqueous solution

If a solution of silver nitrate be added to a solution of potassium cyanide, double decomposition takes place with formation of silver cyanide. But in presence of the excess of the alkali cyanide, the silver cyanide is not precipitated since it is kept in solution as the soluble potassium silver cyanide $KAg(CN)_2$. The reaction may therefore be represented by the equation

$$2KCN + AgNO_3 = KAg(CN)_2 + KNO_3.$$
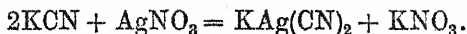
If the addition of the silver nitrate be continued, a point is at length reached at which silver cyanide is precipitated, that is to say, the following reaction begins:

$$KAg(CN)_2 + AgNO_3 = 2AgCN + KNO_3.$$
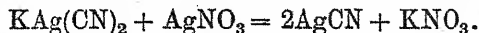
The end-point of the reaction shown in the first equation is determined without the addition of any foreign substance to the solution to serve as an indicator, it is simply that point at which the previously clear liquid becomes slightly turbid as the result of the separation of insoluble silver cyanide. It is clear from what has been stated that the calculation of the amount of potassium cyanide in solution is to be made from the first equation given above; or in other words if n_1 c.c. of the potassium cyanide

solution require the addition of n_2 c.c. of silver nitrate solution containing w grammes of the salt dissolved in each cubic centimetre, the weight x of potassium cyanide per cubic centimetre is calculated from the equation

$$\frac{n_1 x}{n_2 w} = \frac{2 \times 65}{170}.$$

Owing to the highly poisonous character of hydrocyanic acid, the vapour of which is continuously being given off from solutions of potassium cyanide in consequence of hydrolysis, it is not advisable to measure out potassium cyanide solution in an ordinary pipette; it is much safer to make use of an automatic delivery pipette which obviates the necessity of inhaling the poisonous vapour.

Determination of silver by means of standard sodium chloride

The examples which we have given of the application of silver nitrate to the determination of chlorides, bromides, and iodides in solution may clearly be applied in the reciprocal way. That is to say, silver in its soluble salts may be quantitatively precipitated by means of solutions of any halide. For this purpose use is almost invariably made of sodium chloride, and indeed the determination of silver by means of standard sodium chloride solution is one of the oldest volumetric processes, and is still sometimes employed in the wet-assay of silver. For this latter purpose it is usual to determine the end-point by ascertaining when precipitation is complete, the procedure being rendered very accurate by completing the precipitation with a solution of sodium chloride of one-tenth of the strength of that used for precipitating the main quantity of the metal. Silver in acid solution may be determined with a high degree of accuracy with the aid of an adsorption indicator such as phenosafranine or tartrazine (see pp. 150–158).

CHAPTER VII

THE DETERMINATION OF SILVER IN ACID SOLUTION BY STANDARD AMMONIUM THIOCYANATE

If a solution of ammonium or potassium thiocyanate be added to a solution of silver nitrate, double decomposition takes place with precipitation of the sparingly soluble silver thiocyanate AgCNS, thus

$$AgNO_3 + NH_4CNS = AgCNS + NH_4NO_3.$$
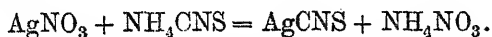
Upon this reaction, Volhard founded an elegant and accurate method for the volumetric determination of silver. Soluble thiocyanates react with ferric salts with formation of the well-known blood red colour of ferric thiocyanate; and consequently the end-point of the reaction is determined with great precision by adding a small quantity of ferric sulphate to the silver solution. As long as the precipitation of the silver thiocyanate is incomplete the solution remains colourless, but as soon as the slightest excess of thiocyanate has been added, the red colour of ferric thiocyanate imparts a permanent red colour to the liquid, which persists when it is shaken.

Preparation of a deci-normal solution of ammonium thiocyanate

Ammonium thiocyanate being a very deliquescent salt, it is very difficult to weigh out the theoretical quantity (7·6 grammes per litre) to make a solution of exactly deci-normal strength. The best plan is to weigh out a little more than the necessary quantity, so as to make up a solution of rather greater than deci-normal strength. The exact strength of this solution is then found by titration with standard silver nitrate solution. The thiocyanate solution may then be diluted so as to be exactly

deci-normal. In carrying out the titrations it is necessary to add a small quantity of nitric acid (free from lower oxides of nitrogen) to the silver solution, in order to decompose any basic ferric salt which would otherwise impart a brown colour to the liquid and thereby render the end-point less sharp than it should be.

If n_1 c.c. of standard silver nitrate containing w grammes of the salt per c.c. require n_2 c.c. of ammonium thiocyanate to complete the reaction, and if x be the weight of ammonium thiocyanate per c.c., we obtain x by solving the equation

$$\frac{n_2 x}{n_1 w} = \frac{\text{equivalent of ammonium thiocyanate}}{\text{equivalent of silver nitrate}} = \frac{76}{170}.$$

Applications

In the previous chapter we have explained the use of standard silver nitrate as a volumetric reagent for the determination of halides. It was also explained that the estimation of silver may be readily effected volumetrically by the use of a standard solution of sodium or potassium chloride. If the end-point is to be found by means of potassium chromate, the liquid undergoing titration and the volumetric precipitant must both be neutral. Ammonium thiocyanate therefore possesses an advantage over sodium chloride as a reagent for the determination of silver, as it is available in the presence of a considerable excess of free acid. Further, it may be employed for the determination of silver in presence of various other metals, including copper up to 70 per cent.; it is therefore of great value in the analysis of silver alloys. As an example we shall describe the procedure for the determination of silver in a silver-copper alloy. A known weight of the alloy (about 0·4 gramme) is dissolved in dilute nitric acid with the aid of a gentle heat, care being taken to avoid loss of liquid by effervescence. After the action has ceased, the contents of the vessel and washings are transferred to a measuring flask and distilled water is added to dilute the contents to the necessary volume. Aliquot portions of the liquid are withdrawn and titrated with standard ammonium thiocyanate solution, a small quantity of a solution of ferric sulphate being added as an indicator.

The quantity of ferric sulphate which is added to a silver

solution which is titrated by ammonium thiocyanate is a matter of some little importance. Too small a quantity should be avoided, as the end-point is sharper in presence of a relatively large quantity of the iron salt. A little practice will soon enable the experimenter to ascertain how much of the indicator to add in order to obtain the best result.

Determination of halides

The determination of chlorides, bromides, or iodides may be effected satisfactorily by means of a standard solution of ammonium thiocyanate by first adding to the solution of the halide a known quantity of silver nitrate solution, which must be in excess, and then determining by residual titration with standard ammonium thiocyanate the amount of silver nitrate remaining unprecipitated. Formerly it was customary to titrate with ammonium thiocyanate in presence of the precipitated silver halide, but it has gradually become recognized that more accurate results are to be obtained by first filtering off the precipitated silver halide, and titrating the filtrate and washings by means of ammonium thiocyanate. The reason of the lower degree of accuracy which is attained by precipitating the silver thiocyanate in presence of the silver halide has been ascribed to the solvent action of ammonium thiocyanate on silver halides.

All of the substances, including the various mixtures the determination of which we have described in the previous chapter, may be determined by the addition of excess of silver nitrate and back-titration with ammonium thiocyanate. In addition, however, the fact that ammonium thiocyanate is available in the presence of free acid renders the method of residual titration by this substance of great availability. The estimation of chlorides which give an acid reaction in consequence of hydrolysis may be effected with satisfactory results.

CHAPTER VIII

ACIDIMETRY AND ALKALIMETRY

Introduction

When aqueous solutions of an acid and a base are allowed to interact, neutralization takes place with formation of a salt and water, according to the general equation

$$\text{Acid} + \text{Base} = \text{Salt} + \text{Water.}$$
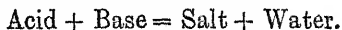
The process of neutralization is not always quantitative, that is to say, there are many cases in which the reaction is reversible to some extent. The reverse reaction to neutralization, that is, the partial decomposition of a salt into free acid and free base, is termed hydrolysis. Hydrolysis, theoretically at least, occurs whenever any salt is dissolved in water, but in those cases in which the salt is the product of a strong acid, such as hydrochloric, and a strong base, such as sodium hydroxide, the degree of hydrolysis is practically infinitesimal. It is otherwise when the salt is the product of an acid and a base, one or both of which is weak. If the acid is weak and the base is strong, the result of hydrolysis is that the salt in solution has an alkaline reaction. Sodium acetate, for example, reacts alkaline in aqueous solution. On the other hand, if the salt is the product of a weak base and a strong acid, the salt will possess an acid reaction in solution in consequence of hydrolysis. Ferric chloride, for example, reacts strongly acid when dissolved in water. The cases in which the salt is derived from a weak base and a weak acid are somewhat more complicated, but for the present purpose it is unnecessary to consider them, since it is impossible to determine a weak acid by means of a weak base satisfactorily, and, as we shall see later, it is never necessary to do so; and generally, in acidimetry and alkalimetry methods are so chosen that hydrolysis comes as little into play as possible.
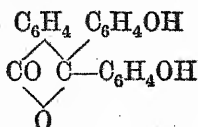
Indicators

In order to determine the point of neutrality, a small quantity of a suitable "indicator" is added to the liquid undergoing titration. The indicators in common use are organic compounds which possess different colours in acid and in alkaline solution. The indicators themselves are feebly acidic or basic substances, and the slightest excess of either acid or base is sufficient to determine the change of colour. The choice of a suitable indicator to be employed in any particular acidimetric or alkalimetric determination is a matter of great importance, and is to be made from a consideration of the properties of the indicator and the relative strengths of the acid and base which are to interact. The theory of indicators will be discussed more fully in the next chapter; for the present purpose it will be sufficient to describe the properties of some of the various indicators in common use and to indicate the reasons for making any particular choice.

Litmus

This substance is one of the oldest indicators, and for many purposes it gives very satisfactory results. It is a substance of a feebly acid nature, and is blue in alkaline and red in acid solution. Neutrality is indicated by a pale lavender tint. This indicator may be employed in the titration of strong acids by strong bases, but it is not a sufficiently feeble acid to give satisfactory results with the weakest organic acids. Litmus is a weaker acid than carbonic acid; in other words, it undergoes a colour change under the influence of carbon dioxide; consequently in titrating alkaline carbonates in presence of litmus, it is necessary to keep the solution of the carbonate at the boiling point in order to expel carbon dioxide from the solution.

Phenolphthalein

Phenolphthalein or dihydroxyphthalophenone is a substance having the formula

$$C_6H_4 \quad C_6H_4OH$$
$$CO \quad C—C_6H_4OH$$
$$O$$

and is so weak an acid as to be almost devoid of acid properties in solution. It is soluble in dilute alcohol, and is red in alkaline but colourless in acid solution. This indicator, on account of its excessively weak acidic properties, is very well adapted for the determination of the weakest organic acids by titration with caustic soda. Phenolphthalein cannot be used for the titration of weak bases like ammonia, for reasons which will be explained in the next chapter, nor may it be used in presence of ammonium salts. This indicator is sensitive to carbon dioxide; if an alkaline carbonate be titrated by a strong acid in presence of phenol-phthalein, neutrality will be indicated when the normal carbonate is converted into the bicarbonate, that is, when the normal carbonate is half neutralized.

Methyl orange

This substance is the sodium salt of dimethylaminoazobenzene-sulphonic acid $SO_3HC_6H_4N_2C_6H_4N(CH_3)_2$. It is soluble in water, and is yellow in alkaline but red in acid solution. Although methyl orange has a sulphonic acid group in the molecule, its indicator properties are determined, not by this acidic grouping, but by the amino grouping: it is indeed a very weak base. Alkaline carbonates and borates can be titrated accurately with strong acids, using this indicator, as it is not sensitive to carbonic and boric acids or to other very weak acids. Methyl orange also yields satisfactory results when weak bases like ammonia are titrated by means of strong acids. In using this indicator it is important to avoid the use of too much of it, one drop of a solution of one-tenth per cent. strength is usually amply sufficient. If too great a quantity be employed the end-point will not be sharply defined. The solution to be titrated should not be too dilute, or else intermediate shades of colour will be obtained as the liquid approaches neutrality. Some persons appear to have considerable difficulty in obtaining satisfactory results with methyl orange, but it must be borne in mind that some eyes are much more sensitive to the colour change than others.

Methyl red

Methyl red, like methyl orange, is also an aminoazo compound, but contains an ortho carboxylic instead of a para sulphonic acid group, and its indicator properties are those of a very weak base. The compound has the formula $COOH . C_6H_4N_2C_6H_4N(CH_3)_2$. It is yellow in alkaline, but violet red in acid solution, and is very well adapted for the titration of weak bases, like ammonia, by strong acids. The colour change is very much more striking than that of methyl orange, and is preferable to the latter indicator on that ground. On the other hand, methyl red is decidedly sensitive to carbon dioxide, and consequently unsuited for the titration of alkaline carbonates by mineral acids.

Paranitrophenol

This substance has the formula $C_6H_4(OH)(NO_2)$. It is sparingly soluble in water, but more soluble in water containing alcohol. In acid solution it is perfectly colourless, but in alkaline solution it is coloured yellow. In general properties this indicator has much in common with methyl orange and methyl red, but is rather more sensitive to carbon dioxide. Like methyl orange, it gives satisfactory results when weak bases are titrated by means of strong acids.

Other indicators

Many other organic compounds which are possessed of different colours in acid and in alkaline solution have been proposed from time to time as indicators for special purposes. It will be sufficient to mention such indicators as lacmoid, phenacetolin, congo red, and rosolic acid, but we must refer the reader to some larger treatise for an account of the properties of these substances.

Standard solutions of acid and alkali

In all acidimetric and alkalimetric work it is obviously necessary to have some one substance of great purity with which to prepare a solution of accurately known strength, and in terms of which all other acid and alkaline solutions may be readily standardized. Various substances have been suggested for this purpose.

Succinic acid $C_2H_4(COOH)_2$ is a substance which may be obtained of a high degree of purity by recrystallization; a solution of this substance may be employed to determine the exact strength of solutions of potassium or sodium hydroxide. Since succinic acid is a weak acid, phenolphthalein must be employed in titrating with this acid. Again sodium carbonate Na_2CO_3 may be obtained in a very pure state by heating the bicarbonate $NaHCO_3$ at a temperature of about 270° C., when water and carbon dioxide are expelled. A standard solution of sodium carbonate may readily be prepared and will serve as a reliable standard for the determination of the strengths of acids such as sulphuric or hydrochloric. Some operators employ borax purified by recrystallization as standard substance. The standardization of acids may also be effected with very satisfactory results by the use of metallic sodium. This metal can readily be obtained in a state of great purity, and for most purposes there is no better method of standardizing acids for volumetric work. The metal is cut into small pieces, each of the order of one gramme in weight, freed from naphtha by pressing between filter paper, and after weighing rapidly to the nearest milligramme, each piece is dropped into a flask containing some rectified alcohol. The metal dissolves with evolution of hydrogen and formation of sodium ethoxide which remains dissolved in the excess of alcohol. The flasks should be held in an inclined position during the solution of the metal to prevent loss of liquid by effervescence. The reaction which takes place is represented by the equation

$$2Na + 2C_2H_5OH = 2C_2H_5ONa + H_2.$$
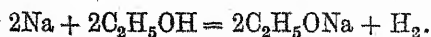
When the metal has been completely dissolved, excess of water is added, when sodium hydroxide is formed according to the equation

$$C_2H_5ONa + H_2O = C_2H_5OH + NaOH.$$
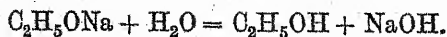
The acid which is to be standardized is then titrated against this solution, litmus being used as an indicator. In standardizing an acid by this method, it is important to weigh the metal rapidly to avoid atmospheric oxidation as much as possible, and it is advisable to make successive determinations by weighing out separate

quantities of sodium, rather than by weighing out one piece of the metal and making up the resulting solution of sodium hydroxide to some definite volume by dilution with water. The sodium must be dissolved in rectified alcohol; methylated spirit must not be used, as the impurities in this substance frequently interfere with the accuracy of the experiment. It is perhaps scarcely necessary to remark that the sodium must not be placed in water directly. In calculating the strength of the acid which is being standardized, it is to be remembered that 23 parts by weight of sodium are equivalent to 36·5 parts by weight of hydrochloric acid, or to 63 parts by weight of nitric acid or to 49 parts by weight of sulphuric acid. There is no necessity to calculate as an intermediate step the weight of sodium hydroxide which is produced from the weighed quantity of metal.

An ingenious method of standardizing acids for volumetric work depending upon the use of Iceland spar was devised some years ago by Orme Masson (*Chem. News*, 1900, p. 73). Iceland spar is calcium carbonate $CaCO_3$ probably in as pure a state as it is possible to obtain any substance. This substance dissolves in hydrochloric acid with formation of calcium chloride and evolution of carbon dioxide according to the equation

$$CaCO_3 + 2HCl = CaCl_2 + H_2O + CO_2.$$
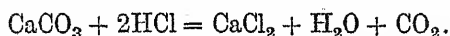
The essential feature of Masson's method of employing this substance for standardizing hydrochloric acid is the use of a known weight of the substance which is always in excess of the amount theoretically required to react with the quantity of hydrochloric acid taken in any particular determination and determining gravimetrically the amount of Iceland spar remaining unacted upon. Small compact fragments of Iceland spar are broken from a large rhomb of the substance and a suitable weight (from 2 to 3 grammes) of the fragments placed in a beaker or other suitable vessel. The beaker with its contents is then carefully weighed. It is to be noted that it is unnecessary to know the weight of either beaker or Iceland spar separately. The measured quantity of hydrochloric acid is then run in from a burette, precautions being taken to guard against loss of liquid by effervescence. The beaker with its contents is then set aside until all

action has ceased. Then after boiling for some time to expel dissolved carbon dioxide from the solution, distilled water being added from time to time to prevent the solution from becoming too much concentrated, the perfectly clear and neutral solution of calcium chloride is decanted off from the excess of Iceland spar which remains in the form of compact fragments. The beaker with the residual solid is then washed out several times with distilled water, dried at a temperature of about 110° C. and weighed. The difference between the initial and final weights of the beaker and Iceland spar is clearly equal to the weight of spar which has been dissolved by the volume of hydrochloric acid taken in the experiment.

Since the molecular weight of calcium carbonate is very nearly 100, it follows that 20 c.c. of a strictly normal acid should dissolve exactly one gramme of Iceland spar. The calculation of the strength of the acid is therefore effected with a minimum of arithmetical work, and further the method has the advantage of extreme simplicity of working, only one volume measurement and two weighings being involved in each determination.

It might be supposed that the method was liable to yield inaccurate results, since it is conceivable that a portion of the residual spar might dissolve in the form of soluble calcium bicarbonate $Ca(HCO_3)_2$. If such were the case, however, the bicarbonate would be decomposed by boiling the solution with formation of the normal carbonate and evolution of carbon dioxide. However, the author states that no turbidity was observable on boiling the solution, and consequently the solid must dissolve entirely as calcium chloride. The experiments which were described certainly indicate that the method is capable of yielding results of extreme accuracy.

An accurate method of preparing standard hydrochloric acid by passing pure dry gaseous hydrogen chloride into water and weighing the amount absorbed was devised by Moody (*Trans. Chem. Soc.*, 1898, p. 658). The hydrogen chloride is generated by heating rock salt with concentrated sulphuric acid, or by dropping strong sulphuric acid into ordinary concentrated hydrochloric acid. In the latter case it is necessary to dry the gas by strong sulphuric acid. The method is a simple one to carry out,

and the results obtained show that it leaves nothing to be desired from the point of view of accuracy.

Having standardized a solution of hydrochloric or sulphuric acid by means of sodium or by other suitable means, we are in a position to prepare standard solutions of all other acids and alkalis*. For example, suppose we have determined the exact strength of a solution of hydrochloric acid and we wish to prepare a quantity of nitric acid of normal strength, all that is necessary is to refer to tables showing the relation between the specific gravity of aqueous solutions of nitric acid and the strength of the acid. Pure concentrated nitric acid is then diluted with water, until the mixture when cold has approximately the correct specific gravity, or preferably a specific gravity very little above that possessed by the normal acid. A solution of caustic soda, the strength of which need not be known, is taken and titrated first by the standard hydrochloric acid, and then by the nitric acid. Since one gramme molecular weight of sodium hydroxide is equivalent to 36·5 grammes of hydrochloric acid and also to 63 grammes of nitric acid, it is a simple matter to calculate the exact strength of the nitric acid. The nitric acid is then cautiously diluted with water and restandardized, and the process repeated until the strength of the acid is exactly normal.

In standardizing the weak organic acids such as acetic and tartaric, it is essential to employ phenolphthalein as the indicator. On the other hand if it is required to prepare a standard solution of ammonia, methyl orange or methyl red must be used in titrating this substance.

The specific gravity of solutions of substances such as sulphuric acid and sodium hydroxide varies in a perfectly regular manner with the concentration of the solution. Consequently the specific gravity is a valuable aid during the preliminary process of preparing a standard solution. In certain cases, however, notably in the case of hydrochloric acid, standard solutions of approximately known strength may be prepared by taking advantage of the

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* It will be clear that in acidimetry and alkalimetry it is only necessary to have *one* standard liquid of each kind. Hydrochloric acid and sodium hydroxide are the reagents usually employed; the former has the advantage over other acids that its strength can be checked independently with reference to silver nitrate.

peculiar phenomena which take place when the aqueous solutions are distilled. It was shown by Roscoe and Dittmar that when a concentrated solution of hydrochloric acid was distilled, the solution gradually became weaker until the strength became constant. On the other hand a dilute solution became more concentrated on distillation, finally becoming of constant strength. The particular strength of hydrochloric acid which behaved on distillation like a pure liquid was found to depend upon the pressure. At a pressure of 760 mm. the constant boiling mixture of hydrochloric acid and water was found to contain 20·2 per cent. of acid. An acid of this strength may be diluted to any desired extent for the preparation of a standard solution (see p. 148).

Determination of the water of crystallization in hydrated sodium carbonate

It has been explained already that alkaline carbonates may be accurately titrated by means of strong acids, methyl orange being used as an indicator. A few grammes of the crystals are weighed out, dissolved in water, and made up to a suitable volume with water. Aliquot portions of the solution are then titrated by means of standard acid. The weight of anhydrous sodium carbonate is then calculated from the equation

$$Na_2CO_3 + 2HCl = 2NaCl + H_2O + CO_2.$$
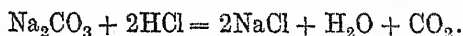
It is clear from this equation that 53 grammes of anhydrous sodium carbonate are equivalent to 36·5 grammes of hydrochloric acid.

If w_1 grammes of the crystals are taken, and it is found by titration that w_2 grammes of anhydrous sodium carbonate are present, the weight of combined water is clearly $(w_1 - w_2)$ grammes. This quantity of water has entered into combination with w_2 grammes of anhydrous sodium carbonate; therefore the weight of water combined with one molecular weight of sodium carbonate is $106 \dfrac{(w_1 - w_2)}{w_2}$ grammes. Dividing this fraction by the molecular weight of water (18), we obtain the number of molecules of water of crystallization in the substance.

*Determination of sodium carbonate and hydroxide when present together*

This estimation is a problem of daily occurrence in alkali works. Formerly it was the usual practice to determine the caustic alkali alone by titration with standard acid after separation of the carbonate by precipitation as insoluble barium carbonate, and then to determine the total alkali by a separate titration. At the present time, the determination of the two constituents is effected by the use of two indicators. If to a solution of a mixture of alkaline carbonate and hydroxide hydrochloric acid be added in presence of phenolphthalein, neutrality will be indicated when the sodium hydroxide has been completely neutralized and the sodium carbonate converted into sodium hydrogen carbonate. In other words the neutralization of the two constituents of the mixture may be represented by the equations

$$NaOH + HCl = NaCl + H_2O,$$

and
$$Na_2CO_3 + HCl = NaCl + NaHCO_3.$$
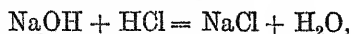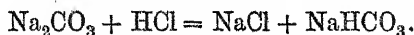
During the titration with phenolphthalein, the tip of the burette should be kept immersed in the liquid to prevent the escape of carbon dioxide. A drop of methyl orange is now added to the liquid, and the titration continued until the reaction is completed. This second reaction consists in the decomposition of the sodium bicarbonate into sodium chloride, carbon dioxide, and water

$$NaHCO_3 + HCl = NaCl + H_2O + CO_2.$$
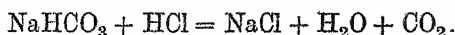
If n_1 c.c. of acid are required to discharge the red colour of the phenolphthalein, and a further n_2 c.c. of acid are required to change the yellow colour of the methyl orange to pink, it is clear that the total alkali is represented by $(n_1 + n_2)$ c.c. of acid. The sodium carbonate alone corresponds to $2n_2$ c.c. of acid, while the sodium hydroxide is represented by $(n_1 - n_2)$ c.c. of acid.

*Determination of sodium carbonate and bicarbonate in a mixture*

From what has been stated in the preceding section, it is clear that it should be possible to determine sodium bicarbonate and normal carbonate by the use of two indicators; and, as a matter

of fact, satisfactory determinations of relatively small quantities of bicarbonate in presence of larger quantities of the normal carbonate may be readily carried out by this method. Phenolphthalein is added to the measured quantity of the solution, and standard hydrochloric or other strong acid is run in until the colour disappears. If v_1 c.c. of acid are added, it is clear that the sodium carbonate is represented by $2v_1$ c.c. of acid. If now a drop of methyl orange be added to the same liquid, and the addition of acid continued until the yellow liquid becomes red, v_2 c.c. of acid run in from the completion of the phenolphthalein titration, that is the total titration is $(v_1 + v_2)$ c.c. of acid, which represents the total alkali. The amount of acid which corresponds to the sodium bicarbonate is clearly equal to the difference between that required for the total alkali and that required for the normal carbonate, or $(v_1 + v_2 - 2v_1)$ c.c. or $(v_2 - v_1)$ c.c.

Determination of ammonium salts

All ammonium salts are decomposed when boiled with excess of solutions of caustic alkalis. Thus when ammonium sulphate is boiled with excess of sodium hydroxide, the following reaction takes place:

$$(NH_4)_2SO_4 + 2NaOH = Na_2SO_4 + 2H_2O + 2NH_3.$$
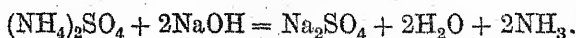
The ammonia being volatile is expelled from the solution. The determination of an ammonium salt may be effected by adding a *known* excess of a *standard* solution of sodium hydroxide, boiling until all the ammonia has been driven off, and then determining by titration with standard sulphuric or other strong acid the amount of alkali remaining in excess. The amount of ammonium salt present is represented by the difference between the amount of sodium hydroxide originally taken and that found by titration after boiling off all the ammonia. Another method of carrying out the determination is to add an arbitrary excess of caustic soda of unknown strength to the ammonium salt and to conduct the decomposition of the ammonium salt in an apparatus for collecting the evolved ammonia in a measured excess of standard acid (Fig. 4). The amount of acid remaining unneutralized is then determined by titration with standard caustic soda. In this case

the amount of ammonium salt present is represented by the difference between the amount of standard acid taken originally and the amount remaining after absorption of the whole of the ammonia resulting from the decomposition of the salt.

It must be borne in mind that all titrations in presence of ammonium salts must be carried out with litmus or preferably with methyl orange or with methyl red. Phenolphthalein must not be used on account of the hydrolysis of the ammonium phenolphthalein salt.
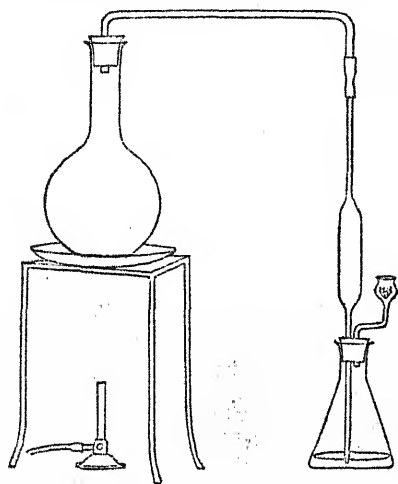

Fig. 4.

Both methods of determining ammonium salts give equally satisfactory results in the absence of substances which introduce complications; but in certain cases, the method of estimating the ammonium salt by collecting the evolved ammonia in excess of standard acid must be employed. For example if it be desired to determine the percentage of the ammonium group in ammonium ferrous sulphate, the addition of caustic soda to this salt is immediately followed by the precipitation of ferrous hydroxide which would make titration with acid impossible. On the other hand the determination may be carried out with satisfactory results by the second method.

Determination of two acids in a mixture when the total acid is known

The general principle of the method of indirect analysis depending upon the difference between the equivalent weights of the two constituents has been already described in connexion with the determination of two halides by means of standard silver nitrate (Chapter VI). It is obvious that a precisely similar procedure should suffice for the determination of two acids such as nitric and sulphuric by means of standard sodium hydroxide, and as a matter of fact satisfactory determinations of this kind may be readily made.

If we have a mixture of nitric acid and sulphuric acid of such a strength that one litre of the solution contains w_1 grammes of the mixed acids, and we find by titration that w_2 grammes of sodium hydroxide are required for complete neutralization of a litre of the solution, the weights of the two acids are found by solving the simultaneous equations

$$x + y = w_1 \quad \dots\dots\dots\dots\dots (1),$$

$$\frac{40x}{63} + \frac{40y}{49} = w_2 \quad \dots\dots\dots\dots\dots (2),$$

where x and y denote respectively the weights of nitric and sulphuric acid in grammes dissolved in one litre of the mixture.

The theory of the errors involved in indirect determinations of this kind has been already discussed (Chapter VI), and it is clear that the method is open to the objections there indicated.

In the example which we have discussed any indicator may be employed for determining neutrality since both of the constituents of the mixture are strong acids, and caustic soda is a strong base. On the other hand if either or both of the acids to be determined are weak, phenolphthalein must be used as the indicator.

Determination of orthophosphoric acid

This substance belongs to the group of acids of medium strength. The formula of the acid, H_3PO_4, and the fact that it gives rise to three series of salts, characterize it as a tribasic acid. On the other hand, its behaviour on neutralization by caustic soda

in presence of various indicators is somewhat peculiar. If the acid be neutralized by sodium hydroxide in presence of methyl orange, neutrality will be indicated when only one of the three hydrogen atoms has been replaced by sodium, dihydrogen sodium phosphate being formed according to the equation

$$H_3PO_4 + NaOH = NaH_2PO_4 + H_2O.$$
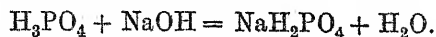
On the other hand if phenolphthalein be employed as indicator in the titration of this acid, the addition of sodium hydroxide may be continued until two equivalents of the base have been added to each molecule of phosphoric acid; in other words neutrality will be indicated when hydrogen disodium phosphate has been formed as indicated by the equation

$$H_3PO_4 + 2NaOH = Na_2HPO_4 + 2H_2O.$$
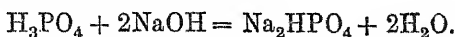
The theoretical explanation of this behaviour on neutralization will be given in the next chapter; for the present purpose all that it is necessary to bear in mind is that the calculation of the amount of acid present from the titration is to be made from the first equation if methyl orange is employed as the indicator, and from the second equation if the determination is carried out in presence of phenolphthalein.

Determination of citric acid

Citric acid is a tribasic acid of the formula

$$CH_2 . COOH$$
$$|$$
$$C(OH)COOH$$
$$|$$
$$CH_2 . COOH$$
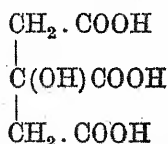
It crystallizes with one molecule of water. Like phosphoric acid it behaves differently with different indicators on neutralization with sodium hydroxide. With methyl orange citric acid behaves as a monobasic acid, neutrality being indicated when the formation of the monosodium citrate has taken place. On the other hand, when phenolphthalein is employed as indicator, the trisodium salt of the acid is formed; that is, the substance then behaves as a tribasic acid.

Determination of borax

Borax is a substance of the formula $Na_2B_4O_7$. It is the salt of an extremely weak acid and a strong base. Borax crystallizes with ten molecules of water, and is moderately soluble in water. The aqueous solution reacts strongly alkaline as a result of hydrolysis, and may be titrated by means of a strong acid using methyl orange as an indicator, when free boric acid is formed, but owing to the very feebly acid character of this substance the end-point with methyl orange is quite satisfactory. The neutralization may be expressed by the equation

$$2HCl + Na_2B_4O_7 + 5H_2O = 2NaCl + 4H_3BO_3.$$

If now to this liquid a little phenolphthalein be added, the free orthoboric acid may be titrated by means of sodium hydroxide. The end-point however is quite unsatisfactory, the red colour appearing before the whole of the free boric acid has been neutralized. It has been found, however, that if a moderately large quantity of glycerol or mannitol be added to the liquid undergoing titration, then the end-point does really represent the point at which all the boric acid has been neutralized. The reaction is represented by the equation

$$4H_3BO_3 + 4NaOH = 4NaBO_2 + 8H_2O,$$
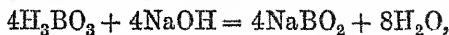
sodium metaborate being formed.

It will be observed from what has been stated that borax may be titrated first as a base and then as an acid after liberation of the orthoboric acid by a strong acid, and exactly double the molecular proportion of sodium hydroxide is required for neutralization as acid to the quantity of hydrochloric acid which is required for neutralization as base. The extremely weakly acid properties of boric acid are shown by the fact that when neutralized by caustic soda in presence of phenolphthalein, the end-point is reached while a relatively considerable proportion of the acid remains unneutralized. Certain polyhydric alcohols such as glycerol stimulate the activity of the boric acid by the formation of stronger complex acids which can be titrated accurately with sodium hydroxide, using phenolphthalein as indicator.

The sodium hydroxide which is employed for titrating borax must be absolutely free from carbonate, in the first place because phenolphthalein is being employed as indicator, and secondly because sodium carbonate itself reacts with boric acid in accordance with the equation

$$Na_2CO_3 + 4H_3BO_3 = Na_2B_4O_7 + CO_2 + 6H_2O.$$
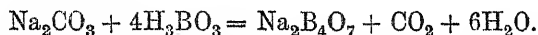
In this equation four atoms of boron are equivalent to two atoms of sodium, whereas in the previous equation four atoms of boron are equivalent to four atoms of sodium.

Baryta water as standard alkali

For many purposes it is useful to employ a solution of barium hydroxide as a standard alkaline volumetric reagent. This substance is a strong base and is on that account well adapted for the titration of weak organic acids such as succinic in presence of phenolphthalein. The solution should be of deci-normal strength, since stronger solutions are liable to separation of the solid on account of its limited solubility. Baryta water must be protected very carefully from atmospheric carbon dioxide since this gas causes precipitation of insoluble barium carbonate, which would of course alter the strength of the solution. The solution should therefore be preserved in a bottle in direct connexion with the burette, and a soda lime tube must be inserted in the upper part of the vessel to absorb atmospheric carbon dioxide. Notwithstanding these precautions the solution will require frequent titration with standard acid. Further the use of baryta water is open to the objection that sooner or later some barium carbonate is precipitated in the burette and "fouling" of the glass surface takes place.

CHAPTER IX

THE THEORY OF INDICATORS

It is difficult, if not impossible, to define in a brief formula what an acid or a base is; that is to say, to give such definitions of these two classes of substances as would be intelligible to anyone who had no practical acquaintance with them. The hypothesis of electrolytic dissociation has greatly facilitated the precise characterization of these classes of compounds. In terms of this hypothesis, acids are defined as compounds of hydrogen which produce free hydrogen ions in aqueous solution; the acidic properties of the solution being associated with the presence of hydrogen ions. Similarly the alkaline properties associated with solutions of basic substances are, in terms of the ionic theory, due to the presence of free hydroxyl ions.

Indicators are to be regarded either as weak acids or as weak bases, and Ostwald has applied the ionization hypothesis in a simple and ingenious manner to the elucidation of the behaviour of the indicators which are commonly applied to determine neutrality. This theory depends upon the following points in the ionic theory:

(1) Strong acids and strong bases are largely dissociated electrolytically in solution; that is, their solutions contain a large quantity of hydrogen or of hydroxyl ions respectively.

(2) Weak acids and weak bases are but little dissociated; their solutions contain only small quantities of hydrogen or of hydroxyl ions respectively.

(3) Salts which are the product of either a weak base and a strong acid, or of a strong base and a weak acid, are largely dissociated in aqueous solution.

(4) Salts belonging to the types considered in (3) undergo hydrolytic dissociation as well as ionization. Hydrolysis is still more pronounced in the cases of salts derived from weak bases and weak acids. Such solutions contain besides small quantities

of the cations and anions of the salt, a large quantity of the salt
in the form of undissociated acid and base.   For example phenol-
phthalein is an extremely weak acid.   The addition of ammonium
hydroxide to this substance results in the formation of the
ammonium salt of phenolphthalein, a salt which is hydrolysed
to a very considerable extent.   Denoting, for shortness, phenol-
phthalein by HPh, the formation of ammonium phenolphthalein
may be represented by the equation

$$NH_4OH + HPh \rightleftharpoons NH_4Ph + H_2O.$$
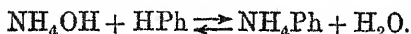
Whether ionization or hydrolysis will predominate in any
particular case depends of course on the conditions of equilibrium.

(5) If ions, which are capable of giving rise to a feebly
dissociated compound, happen to come together in solution,
that compound is formed.   For this reason, and also for another
reason which will appear presently, the already small electrolytic
dissociation of a weak acid or a weak base is reduced to a further
extent if excess of hydrogen ions or of hydroxyl ions be added to
the solution.   In the case of a weak organic acid, such as acetic
acid, there is a definite equilibrium between the undissociated part
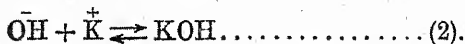of the molecule and the ions

$$CH_3COOH \rightleftharpoons \overset{+}{H} + CH_3\overset{-}{CO_2}.$$
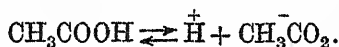
This equilibrium is regulated by the law of mass action as repre-
sented by the equation

$$a \cdot b = k \cdot c,$$

where a denotes the concentration of the hydrogen ions, b the
concentration of the anion, c the concentration of the undissociated
molecule, and k the equilibrium constant.

If, now, a strong acid, in other words if excess of hydrogen ions,
be added to the solution, the equilibrium will be disturbed with
the result that the ionization of the weak acid will be suppressed.
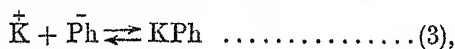
We can now discuss the behaviour of an indicator towards acids
and bases from the standpoint of this theory.   Denoting as before
phenolphthalein by the symbol HPh we may write the equilibria

$$\overset{+}{H} + \overset{-}{Ph} \rightleftharpoons HPh \dots\dots\dots\dots\dots (1),$$

$$\overset{-}{OH} + \overset{+}{K} \rightleftharpoons KOH \dots\dots\dots\dots\dots (2).$$

The result of adding potassium hydroxide to the phenolphthalein is to form water by the union of the hydrogen and hydroxyl ions. Now water is dissociated electrolytically only to an exceedingly small extent. The result is that hydrogen ions disappear from the solution. The equilibrium represented by equation (1) is destroyed and consequently, in order to establish equilibrium again, more molecules of phenolphthalein must undergo ionic dissociation. The result of this is that the concentration of the phenolphthalein ions is increased.

The solution is now coloured red, so we conclude that the red colour is to be connected with the phenolphthalein ion. The condition of the solution is now represented by

$$\overset{+}{K} + \overset{-}{Ph} \rightleftharpoons KPh \ \dots\dots\dots\dots (3),$$

$$\overset{-}{OH} + \overset{+}{K} \rightleftharpoons KOH \ \dots\dots\dots\dots (4).$$

It is clear that in consequence of the addition of a sufficient quantity of caustic potash, the hydrogen ions of the phenolphthalein and the hydroxyl ions of the potassium hydroxide combine to form water so that instead of the weak, feebly dissociated acid phenolphthalein, we now have the potassium salt of phenolphthalein which, being a salt derived from a weak acid and a strong base, is highly ionized.

If now we add a strong acid, such as hydrochloric, to the red alkaline solution, the excess of alkali is neutralized according to the equation

$$\overset{-}{OH} + \overset{+}{K} + \overset{+}{H} + \overset{-}{Cl} \rightleftharpoons \overset{+}{K} + \overset{-}{Cl} + H_2O \ \dots\dots (5).$$

Finally, if excess of hydrochloric acid be added, in other words if an excess of hydrogen ions be added to the solution, the solution will contain two kinds of ions which are capable of producing a compound which is very slightly ionized in solution, viz. phenolphthalein. The formation of phenolphthalein may be represented by the equation

$$\overset{+}{K} + \overset{-}{Ph} + \overset{+}{H} + \overset{-}{Cl} \rightleftharpoons \overset{+}{K} + \overset{-}{Cl} + HPh \dots\dots (6),$$
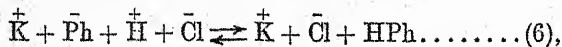
or more simply thus

$$\overset{-}{Ph} + \overset{+}{H} \rightleftharpoons HPh \dots\dots\dots\dots (7).$$

Since phenolphthalein ions disappear from the solution, in order to restore the equilibrium represented by equation (3) the process must take place from right to left, *i.e.* the small quantity of undissociated potassium phenolphthalein dissociates into its ions, and the phenolphthalein ions combine with the excess of hydrogen ions of the hydrochloric acid to form undissociated phenolphthalein. In other words the end of the reaction consists in a complete reformation of the undissociated phenolphthalein molecule, so that we return to the condition of equilibrium represented in equation (1)

$$\overset{+}{H} + \overset{-}{Ph} \rightleftarrows HPh$$
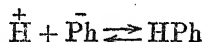
and consequently to a colourless solution.

What has been stated with regard to strong acids such as hydrochloric is equally true of weak organic acids, since they are sufficiently strong to suppress the ionization of phenolphthalein so that a colourless solution is the result. Now experiment shows that strong bases such as potassium and sodium hydroxides may be determined with great accuracy by titration with strong acids, but weak bases such as ammonia cannot be determined in this way. The explanation of this is given by the theory which we have discussed. After the strong base has been neutralized by the strong acid, the solution contains the ions of potassium and phenolphthalein, which result from the dissociation of the potassium salt of phenolphthalein; and an extremely small concentration of hydrogen ions is required in order to suppress the phenolphthalein ions into undissociated phenolphthalein, that is to decolorize the solution.

But if the base is a weak one like ammonia, the strongly hydrolysed salt ammonium phenolphthalein is formed. The red colour of the solution is due to a large excess of ammonia which suppresses hydrolysis. If this excess of ammonia is removed by titration with acid, a point is at length reached when there is not enough ammonia to suppress the hydrolysis of the salt. The solution does not contain the ions of phenolphthalein but colourless undissociated molecules of phenolphthalein; in other words the red colour is discharged before all the ammonia is neutralized by the acid which is employed in the titration.

Basic indicators

The behaviour of a basic indicator, such as methyl orange, may be discussed on similar lines. A compound of this kind may be represented as giving rise to hydroxyl ions by writing an equation such as the following:

$$\text{MeOH} \rightleftharpoons \overset{+}{\text{Me}} + \overset{-}{\text{OH}} \quad \dots\dots\dots\dots(8).$$
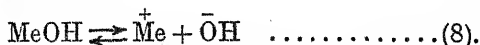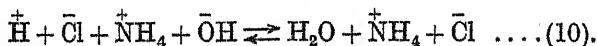
This ionization takes place to a very small extent, and may be suppressed almost completely by adding a small concentration of hydroxyl ions, *e.g.* by the addition of ammonia, which ionizes as follows:

$$\text{NH}_4\text{OH} \rightleftharpoons \overset{+}{\text{NH}}_4 + \overset{-}{\text{OH}} \quad \dots\dots\dots\dots(9).$$
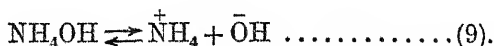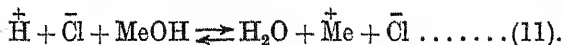
The result is the formation of undissociated methyl orange which is yellow in colour.

If now a strong acid, such as hydrochloric acid, be added to the liquid, the hydrogen ions of the acid will at once combine with the hydroxyl ions to form water, thus:

$$\overset{+}{\text{H}} + \overset{-}{\text{Cl}} + \overset{+}{\text{NH}}_4 + \overset{-}{\text{OH}} \rightleftharpoons \text{H}_2\text{O} + \overset{+}{\text{NH}}_4 + \overset{-}{\text{Cl}} \quad \dots.(10).$$

Further addition of acid will result in the withdrawal of more hydroxyl ions, and in order that this process may take place, there must be further ionization of the indicator in order to keep up the supply of hydroxyl ions

$$\overset{+}{\text{H}} + \overset{-}{\text{Cl}} + \text{MeOH} \rightleftharpoons \text{H}_2\text{O} + \overset{+}{\text{Me}} + \overset{-}{\text{Cl}} \quad \dots\dots(11).$$

The result is that the colour of the cation of methyl orange, which is red, becomes apparent.

Weak acids, such as acetic acid, are not capable of sharp titration with indicators of this kind, because being feebly ionized, they do not supply a sufficient concentration of hydrogen ions in order to determine the ionization of the indicator at the moment when all the hydroxyl ions of the base have been removed. A considerable quantity of the acid is required.

Colour and ionization

At the present time, strong electrolytes are considered by many chemists to be almost completely ionized at all concentrations.

The blue colour of salts such as cupric nitrate and cupric sulphate, which persists from concentrations ranging from saturated solutions up to very great dilution, is a property of the hydrated cupric ion. The change of colour which solutions of cupric chloride suffer by gradual dilution with water from green to blue was formerly explained by assuming that the undissociated molecules of the salt gradually ionized with progressive dilution. The production of a brown colour on adding concentrated hydrochloric acid was likewise explained by the suppression of the ionization of the cupric halide by the large excess of chlorine ions present. The modern theory of total ionization offers a different explanation of these phenomena. In concentrated solution, cupric chloride is probably present as an auto-complex, $\overset{++}{Cu}[\overline{Cu}\overline{Cl}_4]$, the complex anion $[\overline{Cu}\overline{Cl}_4]$ having a brown colour. On dilution with water, the complex anions are decomposed into the blue hydrated cupric cations and simple chlorine anions.

A change of colour is to be interpreted as due to a change of constitution of the ions. The simple ionization theory of indicators, as expounded by Ostwald, must therefore be regarded as incomplete. Apart from ionic changes, the change of colour of indicators in acid and alkaline solution must be regarded as due to a change of constitution of the indicator. At the present time, indicators are no longer regarded as simple acids or bases, which ionize directly as Ostwald assumed, but as compounds, electrically neutral in themselves, which can undergo isomeric changes into acids or bases, and these latter subsequently ionize. Such compounds have been termed pseudo acids or pseudo bases by Hantzsch.

Pseudo acids and pseudo bases

Of the many compounds which are known as neutral non-electrolytes which contain hydrogen, not directly replaceable by metals, but which are capable of undergoing isomeric change into acids, the nitrophenols are perhaps the best examples for discussing indicators, considered as pseudo acids. The nitrophenols have been very carefully investigated by Hantzsch and his collaborators, who have found that these compounds give rise to two series of ethers, one derived from the true nitro compound such as
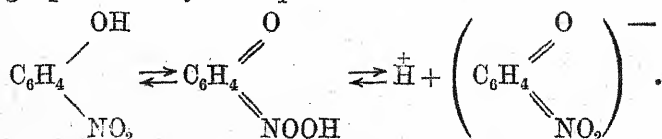
$C_6H_4 \begin{smallmatrix} OH \\ \\ NO_2 \end{smallmatrix}$, which are colourless, the other derived from the

isomeric acid form $C_6H_4 \begin{smallmatrix} O \\ \\ NOOH \end{smallmatrix}$, and are intensely red in colour.

The derivatives of the true nitrophenol are considered to possess a benzenoid structure, while the compounds derived from the acidic isomer are regarded as quinonoid in constitution. The indicator properties of paranitrophenol are well known, the compound being yellow in alkaline and colourless in acid solution. In the solid state, paranitrophenol is colourless, and therefore exists entirely, or very nearly, as the true nitro compound. In solution, there is an appreciable yellow colour, indicating the formation of a perceptible quantity of the isomeric acid form. This latter compound then undergoes electrolytic dissociation into hydrogen

ions, and the coloured anions $\left(C_6H_4 \begin{smallmatrix} O \\ \\ NO_2 \end{smallmatrix} \right)^{-}$, the equilibrium

being represented by the equation:

$$C_6H_4 \begin{smallmatrix} OH \\ \\ NO_2 \end{smallmatrix} \rightleftarrows C_6H_4 \begin{smallmatrix} O \\ \\ NOOH \end{smallmatrix} \rightleftarrows \overset{+}{H} + \left(C_6H_4 \begin{smallmatrix} O \\ \\ NO_2 \end{smallmatrix} \right)^{-}.$$

The strong yellow colour of paranitrophenol in alkaline solution is presumably due to the formation of water by the union of the hydrogen ions derived from the acidic isomer with the hydroxyl ions derived from the base, resulting in the presence of the strongly

coloured anion $\left(C_6H_4 \begin{smallmatrix} O \\ \\ NO_2 \end{smallmatrix} \right)^{-}$. In acid solution, these changes

are reversed, the colour therefore is discharged due to the formation of the true nitrophenol.

This modification of the theory of indicators involves simply the substitution of the equilibrium mixture of the tautomeric forms, for that of the simple undissociated molecule, in the discussion of the ionic equilibria in acid and alkaline solution with the corresponding changes of colour. It is to be noted, however, that the colours of the neutral and acidic isomers must be different, and that the acidic anion must be similar in colour to that of its undissociated molecule. Similar considerations apply to the discussion of basic indicators as pseudo bases.

As the changes of colour of indicators in acid and alkaline solution involve tautomeric changes as well as ionic reactions, it is clear that these tautomeric changes must be rapid, in order that the indicator may work sharply. Ionic reactions are practically instantaneous, but tautomeric changes are not necessarily so, and some indicators, cyanine for example, are unsatisfactory on this account, that is, the change of colour is too slow for convenience in titration.

Hydrogen ion concentration

It has been shown by Ostwald that the ionization of weak electrolytes is strictly in accordance with the law of mass action, and various lines of enquiry have shown that pure water is dissociated electrolytically into hydrogen and hydroxyl ions, to the extent that ten million litres of water contain 1 gramme of hydrogen and 17 grammes of hydroxyl in the ionic condition at the ordinary temperature. This result may be expressed thus:

$$C_H \times C_{OH} = 10^{-14}.$$

As the concentrations of the hydrogen ions and hydroxyl ions are equal, $C_H = C_{OH} = 10^{-7}$, that is the concentration of hydrogen ions is 10^{-7} gramme ion per litre in pure water at 25° C. A more convenient way of expressing this result is to take the logarithm of this number, omitting the negative sign, and write $pH = 7$.

The 'pH' system of expressing the degree of acidity of liquids is of great importance, and has come into general use for many purposes. It will be clear that a strictly neutral liquid is to be defined as one in which the concentration of the hydrogen ions

is equal to that of the hydroxyl ions, the value being a pH of 7. When the value of pH is numerically less than 7, the liquid has an acid reaction, while values of pH which are greater than 7 denote alkalinity. For purely analytical purposes, the concentration of acids and alkalies is usually expressed in terms of normality. This mode of expression, however, takes no cognizance of the actual *ionic* concentration of the acid or base. A given volume of deci-normal sodium hydroxide will neutralize an equal volume of either deci-normal hydrochloric acid or acetic acid, but the ionic concentration of the two acids is very different. Both acids in deci-normal concentration contain the same total of acid hydrogen, which is represented by the sum of the actual and the potential hydrogen ions. In the strong acid the actual ions are present in very high concentration, in the weak acid their concentration is very small. Deci-normal hydrochloric acid has a pH value of the order of 1·0, while deci-normal acetic acid, which is ionized to the extent of about 1 per cent. at the ordinary temperature, has a pH value of 2·87.

In titrating an acid by a base, the object in view is to determine when equivalent quantities of the acid and base are present together. The determination of the 'end point' of the reaction is therefore the determination of the 'equivalent point.' Now the equivalent point is only identical with the strictly neutral point, *i.e.* with a pH value of 7 when a strong acid is neutralized by a strong base, because the salt which is formed will not be hydrolysed. When a weak acid is neutralized by a strong base, the equivalent point will have a pH value numerically greater than 7, because of the alkalinity due to the hydrolysis of the resulting salt. For similar reasons, the equivalent point of the neutralization of a weak base by a strong acid corresponds to a pH value less than 7, since the resulting salt will be acid in consequence of hydrolysis. Now indicators do not, in general, register absolute neutrality, what they do is to change their colour over some definite pH range which is characteristic of the particular indicator. The following examples may be quoted by way of illustration.

Indicator	Colour change	pH range
Methyl violet	Yellow–green	0·1– 1·5
	Green–violet	1·5– 3·2
Methyl orange	Red–yellow	3·0– 4·4
Methyl red	Red–yellow	4·4– 6·2
Paranitrophenol	Colourless–yellow	5·0– 7·0
Litmus	Red–violet–blue	5·0– 8·0
Phenolphthalein	Colourless–red	8·2–10·0
Thymolphthalein	Colourless–blue	9·3–10·5

It will now be clear why methyl orange is suitable for the titration of weak bases by strong acids, and why phenolphthalein is to be used when weak acids are titrated by strong bases.

Since the equilibrium between the undissociated molecule of a weak acid and its ions is regulated by the law of mass action, the relation may be expressed thus:

$$\frac{\text{Concentration of hydrion} \times \text{concentration of anion}}{\text{Concentration of undissociated molecule}} = K,$$

where K is known as the dissociation constant of the acid. It is most important to notice that it is only weak acids which have a dissociation constant, since the ionization of strong electrolytes does not follow the law of mass action. The hydrogen ion concentration of a solution of a weak acid can be calculated from the dissociation constant in the following simple manner.

Since the concentrations of the hydrion and of the anion are equal, and since the concentration of the undissociated acid is considerable in comparison with that of the ions, we may write

$$C_H = \sqrt{K \times C_{acid}},$$

where C_{acid} denotes the concentration of the acid in molecules per litre. Applying this equation to calculate the hydrogen ion concentration of a deci-normal solution of acetic acid, $K = 1\cdot8 \times 10^{-5}$ at 18° C., and $C_{acid} = 0\cdot1$; hence $C_H = 1\cdot34 \times 10^{-3}$, and therefore $pH = 2\cdot87$.

Buffer mixtures

Although weak acids and weak bases are feeble electrolytes, salts which are derived either from weak acids and strong bases, or from strong acids and weak bases, are good electrolytes. Sodium

acetate and ammonium chloride, for example, are good electrolytes. If some sodium acetate be added to a solution of acetic acid, the small concentration of hydrogen ions of the acid is further reduced by increasing the concentration of acetate ions. For similar reasons, the small concentration of hydroxyl ions in a solution of ammonia is diminished if an ammonium salt such as ammonium chloride be added to the liquid.

It is instructive to compare the pH values of hydrochloric acid with those of acetic acid for various concentrations. The following figures have been published by Michaelis:

Hydrochloric acid.

Concentration	N	$0.1N$	$0.01N$	$0.001N$
pH	0.10	1.076	2.022	3.013

Acetic acid.

Concentration	N	$0.1N$	$0.01N$	$0.001N$
pH	2.366	2.866	3.366	3.866

It will be observed that dilution from normal to milli-normal concentration has an enormous effect upon the pH of hydrochloric acid, but comparatively little effect upon that of acetic acid. Further, normal acetic acid is comparable with centi-normal hydrochloric acid, and deci-normal acetic acid with milli-normal hydrochloric acid as regards hydrogen ion concentration. Further if 1 c.c. of normal sodium hydroxide solution be added to 1 litre of milli-normal hydrochloric acid, the acid will be completely neutralized, the resulting solution having a pH value of 7. On the other hand, if 1 c.c. of normal sodium hydroxide be added to 1 litre of deci-normal acetic acid, the acid will not be nearly neutralized, because of the large reserve of potential hydrogen ions in the acetic acid, actually the pH of the solution will change from 2.87 to 3.80. Again, if equivalent solutions of acetic acid in pure water and in a solution of sodium acetate be compared by adding the same amount of sodium hydroxide solution to each, it will be found that the hydrogen ion concentration of the pure acid solution will change much more than that of the mixture of acetic acid and sodium acetate. This resistance to change of hydrogen ion concentration shown by mixtures of weak acids and their salts and by weak bases and their salts, is known as buffer action, the mixtures being termed buffers or regulators.

Titration curves

The course of the change of hydrogen ion concentration during the actual process of titration is shown most conveniently by the aid of neutralization curves, the ordinates of which represent the number of cubic centimetres of acid or base added for the neutralization of a given quantity of base or acid, while the abscissae represent hydrogen ion concentration measured on the pH scale. Fig. 5
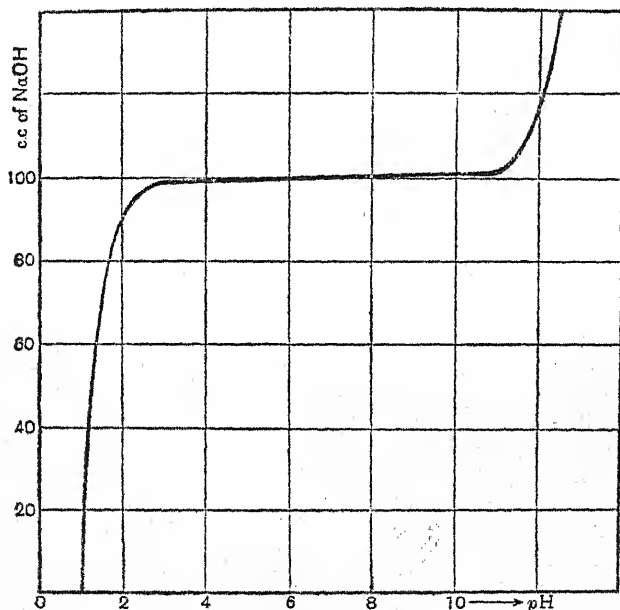

Fig. 5. Neutralization of 100 c.c. of $N/10$ HCl by $N/10$ NaOH.

represents the neutralization of deci-normal hydrochloric acid by deci-normal sodium hydroxide. In Fig. 6, curve I indicates the neutralization of acetic acid by sodium hydroxide, while curve II shows the neutralization of ammonia by hydrochloric acid, the acids and bases being present in deci-normal concentration.

It will be noted that the titration curve of hydrochloric acid by sodium hydroxide is characterized by a horizontal portion, which extends from a pH value of 3 to 11. This considerable range explains why accurate results are obtainable when indicators

which differ so widely in their pH range as methyl orange and phenolphthalein are used in titrating strong acids by strong bases. A comparison of curves I and II in Fig. 6 with Fig. 5 shows that the horizontal part is much shorter. Further, the mid-point of
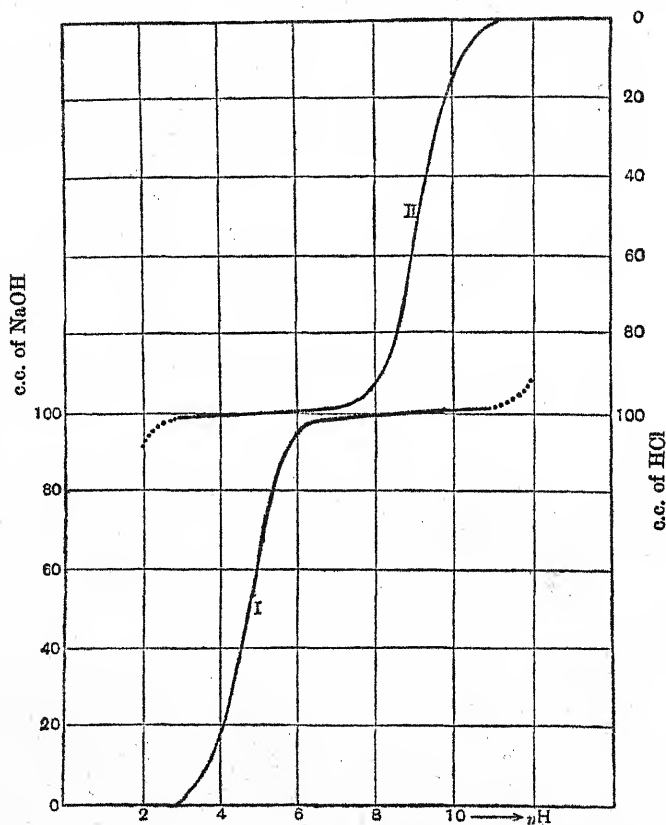

Fig. 6. Curve I. Neutralization of 100 c.c. of N/10 CH$_3$COOH by N/10 NaOH.
Curve II. Neutralization of 100 c.c. of N/10 NH$_4$OH by N/10 HCl.

the horizontal part of the curve in Fig. 5 occurs at the strictly neutral point (pH $=$ 7), because the salt formed, sodium chloride, is not hydrolysed.   The mid-point of the horizontal part of curve I (Fig. 6) occurs at a pH value of 8·9, while that in curve II coincides with a pH value of 5·1.   The relative positions of these two curves with respect to the strictly neutral point is to be explained by

the occurrence of alkaline hydrolysis resulting when equivalent quantities of acetic acid and sodium hydroxide are present together in solution, and of acid hydrolysis resulting from the presence of equivalent quantities of ammonia and hydrochloric acid. The buffering action of the salts formed by neutralization has the effect of resisting any marked change of hydrogen ion concentration. The shape of curves I and II is therefore intelligible, and it will be clear why the 'equivalent point' for the neutralization of ammonia by hydrochloric acid is realized by employing an indicator such as methyl red (pH range 4·4 to 6·2), and that for the neutralization of acetic acid by sodium hydroxide is attained by using phenolphthalein (pH range 8·2 to 10·0). The use of indicators which differ in pH range from those just named would clearly give rise to inaccurate results, and the order of magnitude of the errors may be judged from the curves.

Titration of polybasic acids

Reference has been made in Chapter VIII to the peculiar phenomena which occur when citric and orthophosphoric acids are neutralized with strong bases. When methyl orange is used as indicator, both function as monobasic acids, that is, the end point of the reaction is shown when only one of the three available hydrogen atoms is replaced by the alkali metal. If, however, phenolphthalein be employed, citric acid behaves as a tribasic acid, and orthophosphoric acid as a dibasic one.

A polybasic acid may be defined as one having two or more ionogenic hydrogen atoms in the molecule; but the acid ionizes in stages, the first stage taking place to a greater extent than the second, and the second to greater extent than the third. Each stage has, in general, a distinct dissociation constant. When neutralized to the extent of the first stage, that is as far as the formation of the primary acid salt, in presence of an indicator which changes colour with a concentration of hydrogen ions corresponding to the concentration of hydrogen ions of the primary salt, the acid will function as though it were monobasic. If, however, an indicator is selected which changes colour at a concentration of hydrogen ions corresponding to that of the secondary or tertiary salt, then the acid will behave as dibasic or tribasic respectively.

The sensitiveness of indicators

The theory of the errors which are involved by the use of indicators in acidimetry and alkalimetry is complicated and difficult, and reference may be made to a monograph by Bjerrum, in which the subject is discussed in detail. In ordinary analytical work, particularly when strong acids are neutralized by strong bases, the titrations are carried to end-points which are within a certain range of hydrogen ion concentrations. If, however, it is desired to titrate to a given hydrogen ion concentration, it is necessary to introduce two terms, which have been defined as the *indicator exponent* and the *titration exponent* respectively.

Since the ionization of a weak acid is regulated by the law of mass action, it follows that when an indicator is one-half ionized, $K = C_H$, that is the dissociation constant of the indicator is equal to the concentration of the hydrogen ions in the solution in which the indicator is one-half ionized. The indicator exponent, $p\text{I}$, is defined as $-\log K$, while the titration exponent, $p\text{T}$, is the value of $p\text{H}$ corresponding to the optimum colour. At the half-way change, it is clear that $p\text{T} = p\text{I}$. Now colour change is to some extent dependent upon the concentration of the indicator in the solution, particularly in the case of the so-called one-coloured indicators, such as phenolphthalein. The indicator exponent of phenolphthalein is approximately 9·7, and the titration exponent of this indicator can actually be varied between the limits of 8·4 and 9·2, according as much or very little of the substance is used. The problem of the so-called two-coloured indicators, such as methyl orange, is greatly complicated by the difference in intensity of colour of the red and yellow forms. In such cases it is perhaps best to titrate as far as the half-way change, in which case the end-point will be nearly, though not exactly, independent of the concentration of the indicator, because we are titrating to a certain fractional change of it. This, however, does not take account of the fact that the neutralization of the indicator does require a certain amount of acid or alkali, and obviously the more of the indicator which is present, the greater will be the amount required. Most indicators of this class are, however, used in such dilute solution that the effect is negligible.

In titrating to a given hydrogen ion concentration, it is obviously of advantage to select an indicator with as small a pH range as possible. Bjerrum has estimated that with careful work it is possible to titrate with an accuracy of $pH = pT \pm 0.3$, and by introducing colorimetric methods the error may be reduced to ± 0.1.

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CHAPTER X

UNCLASSIFIED VOLUMETRIC DETERMINATIONS

In this chapter we shall give an account of methods of determining various substances by methods which cannot readily be classified under any general scheme, although some of them are concerned with oxidation and precipitation reactions. Some of the methods are empirical in the sense that they require approximately uniform conditions of standardizing the reagent and applying it to the particular determination which it is desired to carry out.

Direct titration with potassium iodate

Andrews (*J. Amer. Chem. Soc.*, 1903, **25**, 756) has shown that the reaction between hydriodic and iodic acids, described on p. 46, can be made to take a completely different course if the potassium iodate is added in excess to the potassium iodide in presence of a very high concentration of hydrochloric acid. Under these conditions, the iodine which is liberated during the reaction is oxidized to iodine monochloride, hydrolysis of the latter compound being suppressed by the great excess of hydrochloric acid present:

$$KIO_3 + 2I_2 + 6HCl = KCl + 5ICl + 3H_2O.$$
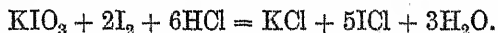
The reaction between potassium iodide and iodate, when carried out in this manner, proceeds as follows:

$$KIO_3 + 2KI + 6HCl = 3KCl + 3ICl + 3H_2O.$$
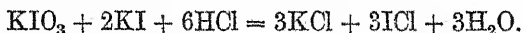
The method of direct titration with potassium iodate has been applied to the determination of free iodine and of iodides, and of other oxidizable substances, the standard solution of potassium iodate being added from a burette to the solution of the oxidizable substance in presence of much concentrated hydrochloric acid. The titrations should be carried out in a stoppered bottle containing a small quantity of chloroform, in which iodine is very soluble, with frequent shaking. The end-point of the reaction is very clearly observed by the violet colour of the iodine dissolved in the chloroform vanishing as the whole of the iodine is oxidized to the colourless monochloride.

The method may be applied to the estimation of thiocyanates, which are oxidized quantitatively to sulphates with liberation of hydrocyanic acid:

$$3KIO_3 + 2KCNS + 8HCl$$
$$= 5KCl + 3ICl + 2H_2SO_4 + 2HCN + H_2O.$$
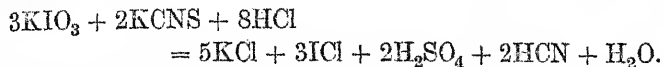
As considerable quantities of concentrated hydrochloric acid have to be present in each titration, usually quantities of the order of 50 c.c. or more are employed when the standard reagent and the substance which is being estimated are present in concentration of the order of deci-normal, it is absolutely necessary to use acid which is free from traces of chlorine. This method has been applied to the determination of thallous salts (Berry, *Analyst*, 1926, p. 137).

Determination of ammonium salts with the aid of formaldehyde

The method depends upon the fact that formaldehyde will react quantitatively in aqueous solution, within certain limits of temperature, with an ammonium salt resulting in the formation of hexamethylene tetramine, $(CH_2)_6N_4$, and the liberation of the acid which was originally present in the ammonium salt. Thus, with ammonium chloride, the reaction proceeds as follows:

$$4NH_4Cl + 6CH_2O = (CH_2)_6N_4 + 6H_2O + 4HCl.$$

Hexamethylene tetramine is a very weak base, much weaker than ammonia, and therefore the hydrochloric acid can be estimated by titration with a strong base using phenolphthalein as indicator.

The best interval of temperature for carrying out the reaction between the ammonium salt and formaldehyde is 50 to 55° C. Below 45° C. the reaction proceeds too slowly, above 60° C. secondary reactions may occur and give rise to complications. The following conditions give excellent results:

Add 4 c.c. of a neutral 20 per cent. solution of formaldehyde to 20 c.c. of the solution of the ammonium salt. Heat the mixture for two minutes at 55° C. then add 20 c.c. of semi-normal sodium hydroxide and wait for two minutes. Finally titrate the excess of alkali with semi-normal hydrochloric acid, using phenolphthalein, and thus determine the quantity of sodium hydroxide neutralized by the acid of the ammonium salt.

Determination of ferric iron by reduction with titanous chloride solution

An aqueous solution of titanous chloride $TiCl_3$ possesses powerful reducing properties, becoming itself oxidized to titanic chloride $TiCl_4$. Thus a solution of ferric chloride is quantitatively reduced to ferrous chloride according to the equation

$$FeCl_3 + TiCl_3 = FeCl_2 + TiCl_4.$$
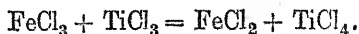
The solution of titanous chloride, which possesses a beautiful purple colour, must be preserved in an atmosphere of hydrogen on account of the great ease with which it undergoes oxidation to titanic chloride which is colourless. The end-point of the reduction of the ferric salt is readily observed by the addition of some ammonium thiocyanate solution, and the addition of the titanous chloride solution is continued until the dark red solution becomes colourless.

Titanous chloride in concentrated solution is now a commercial product, and the standard solution is prepared by boiling about 50 c.c. of the commercial solution with about 100 c.c. of strong hydrochloric acid. The mixture is then diluted with water to about 2·25 litres and preserved in a reservoir which is in connexion with an apparatus for generating hydrogen from zinc and hydrochloric acid. The burette for delivering the liquid is placed in direct connexion with the reservoir.

The iron value of the titanous chloride solution is obtained by dissolving a known weight of pure ferrous ammonium sulphate in water with the addition of a little sulphuric acid and diluting the mixture to a suitable volume. A solution containing about four grammes of the double salt in 200 c.c. will be found of a suitable strength. An aliquot portion of this solution (20 c.c.) is then withdrawn and dilute potassium permanganate solution is added until a faint pink colour remains in the liquid. A moderate excess of ammonium thiocyanate solution is then added, and the titanous chloride solution is then run in from the burette until the red colour of the solution vanishes. Further titrations are carried out in the same way, and the iron value of the titanous

chloride solution determined. The double iron salt contains one-seventh of its own weight of iron. It is clear that it is unnecessary to know the strength of the permanganate solution. The titanous chloride solution can then be employed for the direct determination of an unknown solution of a ferric salt. It is immaterial whether the iron be present in the form of chloride or sulphate, but the presence of free mineral acid is necessary, as otherwise the end-point of the reaction will not be clearly defined. The titanous chloride solution should be titrated against a standard solution of iron at frequent short intervals, even although protected from atmospheric oxygen by being preserved in a hydrogen atmosphere[*].

Determination of the available chlorine in bleaching powder by standard arsenite solution

The chlorine which is present in bleaching powder in the form of hypochlorite when the substance is treated with water may be readily and accurately determined by oxidation of a solution of an alkaline arsenite. The arsenious oxide is converted by the available chlorine into arsenic oxide, 198 parts by weight of arsenious oxide being equivalent to $4 \times 35 \cdot 5$ parts by weight of available chlorine. The method of preparing a standard solution of sodium arsenite has been described in Chapter iv. A suitable quantity of the sample of bleaching powder is weighed out, triturated with water, and made up to a known volume as described in Chapter v. Aliquot portions of the milky liquid are then withdrawn and the standard solution of sodium arsenite allowed to flow in from the burette until the reaction is completed.
end-point of the reaction is determined by means of starch potassium iodide paper employed as an external indicator. This paper is prepared by soaking pieces of filter paper in a solution of starch to which a little potassium iodide has been added. After drying, the paper will be found a sensitive test for certain oxidizing agents such as chlorine. In using starch potassium iodide paper in the determination of bleaching powder, drops of the liquid undergoing titration are removed by means of a glass rod and brought in contact with the paper. As long as any available

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[*] Titanous sulphate solution containing much free sulphuric acid is considerably more stable than the chloride (Russell, *J. Chem. Soc.*, 1926, p. 497).

chlorine remains in the liquid, the paper will be stained a dark colour, but as soon as the reaction has been completed, the paper will remain colourless.

Instead of carrying out the determination with the aid of an external indicator, some chemists prefer to add a measured quantity of the standard solution of sodium arsenite, which must be in excess of the amount required to react with the quantity of bleaching powder taken. The excess of alkaline arsenite is then determined by back titration with standard iodine.

Determination of zinc by means of potassium ferrocyanide

When a solution of potassium ferrocyanide is added to a solution of a zinc salt a sparingly soluble double ferrocyanide of potassium and zinc is precipitated. Upon this reaction a method of determining zinc has been based. This method is of an empirical nature and consequently the conditions of experiment should be adjusted so as to be approximately identical in the different determinations. The ferrocyanide solution is prepared by dissolving about 35 grammes of the crystallized salt in water and diluting the solution to one litre. The solution is standardized by means of a solution of zinc sulphate or chloride prepared by dissolving about one gramme of pure zinc in hot dilute sulphuric or hydrochloric acid. About 10 grammes of ammonium chloride are added to the solution. The solution of the metal is diluted with water and made up to a known volume (200 c.c.). A large excess of free acid should be avoided, but the solution should certainly contain some free acid. Aliquot portions of the solution are then withdrawn and heated to about 90° C. and the solution of potassium ferrocyanide added from a burette until a drop of the liquid when brought in contact with a drop of a solution of uranyl acetate on a spot plate shows a brown colour due to the formation of uranyl ferrocyanide, indicating that a slight excess of ferrocyanide has been added. The titration should not be carried out too rapidly, since the precipitation of the double ferrocyanide of zinc and potassium takes place somewhat slowly. The end-point with uranyl acetate is usually clearly defined in the absence of certain metals such as iron. When care is taken, it is not difficult to obtain

concordant results with this method. The zinc value of the ferro-cyanide solution having been obtained, the standard solution may be employed to determine unknown zinc solutions. Cone and Cady (*J. Amer. Chem. Soc.* 1927, **49**, 356) have modified the method, using diphenylbenzidine as an *internal* indicator.

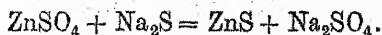### Determination of zinc by means of sodium sulphide solution

Zinc is readily precipitated as sulphide in an alkaline solution, and an excellent volumetric method of determining this element has been based upon this reaction. The standard solution of sodium sulphide is prepared by saturating a strong solution of sodium hydroxide with hydrogen sulphide, and adding more caustic soda until the odour of the gas has been removed. The reactions which occur may be represented by the equations

$$NaOH + H_2S = NaHS + H_2O,$$

and
$$NaHS + NaOH = Na_2S + H_2O.$$
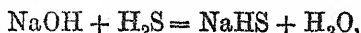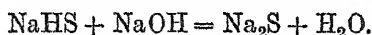
The solution is standardized by means of a solution of a zinc salt prepared by dissolving a known weight of the pure metal in dilute hydrochloric acid and diluting the solution to a suitable volume. The solution of sodium sulphide is titrated against the zinc solution in the following manner. An aliquot portion of the zinc solution is measured out, and a mixture of ammonia and ammonium carbonate added in sufficient quantity to redissolve the precipitate which first forms. The sodium sulphide solution is added from a burette, and drops of the liquid undergoing titration are brought in contact with drops of sodium nitroprusside solution on a spot plate until the presence of a trace of alkaline sulphide in excess is shown by the appearance of a beautiful violet colour with the nitroprusside indicator. The reaction that takes place may be represented by the equation

$$ZnSO_4 + Na_2S = ZnS + Na_2SO_4.$$

The solution of sodium sulphide should then be diluted to a suitable strength for the determinations. It is best to ascertain the zinc value of the standard solution and employ that value rather than to attempt to calculate the strength of the solution from the chemical equation. Some chemists prefer to use an alkaline

solution of lead tartrate instead of sodium nitroprusside as the external indicator, the end-point of the reaction being indicated by the formation of a black precipitate of lead sulphide.   Nickelous chloride has also been employed for the same purpose.

Determination of formaldehyde

Formaldehyde, H.CHO, is a gas which is readily soluble in water.   The product containing about 40 per cent. of formaldehyde is known commercially as formalin, and is much used as a disinfectant.   The substance in dilute aqueous solution may be determined by a number of methods, one of the best depending upon the oxidation of the aldehyde by means of iodine in alkaline solution.   When an aqueous solution of formaldehyde is oxidized by means of iodine in presence of sodium hydroxide, sodium formate and iodide are formed according to the equation

$$H.CHO + I_2 + 3NaOH = H.COONa + 2NaI + 2H_2O.$$
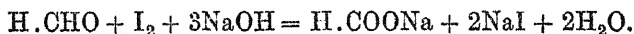
In carrying out the determination, the measured quantity of the dilute aqueous solution of formaldehyde is mixed with a measured quantity of standard iodine solution, which must be in excess. Sodium hydroxide solution is then added until the liquid becomes of a pale yellow colour.   The mixture is allowed to react for ten minutes, and then the solution is acidified with dilute hydrochloric acid to liberate the excess of iodine.   This liberated iodine is then determined by titration with standard sodium thiosulphate solution.   The iodine which has been used up in the oxidation of the formaldehyde is thus determined by difference.

Determination of phosphates

The determination of phosphates may be effected volumetrically by a number of methods, and for general purposes it is very difficult to recommend any one method in preference to another. For ordinary work, the uranium method is perhaps the simplest, and with care it can be made to yield satisfactory results.   This method is, however, an empirical one, and consequently it is important to see that the same conditions of experiment are observed in standardizing the uranium solution as are employed

in carrying out the determination of phosphates in unknown solutions.

The determination of phosphates by precipitation with uranyl nitrate or acetate depends upon the fact that the phosphate radical is precipitated as uranyl phosphate. The precipitation takes place somewhat slowly in the cold, but more rapidly at a higher temperature; a temperature of about $90°$ C. yields good results. The end-point of the reaction is determined by means of potassium ferrocyanide solution on a spot plate, when the presence of the slightest excess of the uranium solution is shown by the appearance of the brown colour of uranyl ferrocyanide. If uranyl nitrate is employed as the standard solution, it is necessary to add sodium acetate to the liquid undergoing titration in order to prevent the possibility of the occurrence of free nitric acid in the solution; with uranyl acetate, the addition of sodium acetate is unnecessary, but if added it will do no harm.

The standardization of the uranium solution may be effected either on pure tricalcium phosphate $Ca_3(PO_4)_2$ or on hydrogen sodium ammonium phosphate $NaNH_4HPO_44H_2O$ (microcosmic salt). The former salt is to be preferred if the uranium solution is required for the determination of phosphoric acid in combination with calcium or magnesium. A suitable quantity, say 10 grammes, of tricalcium phosphate is weighed out and dissolved in a little nitric acid, and the liquid diluted to a litre. If there is any doubt about the purity of the calcium phosphate, the phosphorus should be determined gravimetrically as magnesium pyrophosphate.

The uranium solution is prepared by dissolving about 35 grammes of either the acetate or the nitrate in water and diluting the solution to one litre. This solution is standardized by measuring out a suitable volume of the standard phosphate solution, heating to about $90°$ C., and titrating with the uranium solution until a drop of the liquid when brought in contact with a drop of potassium ferrocyanide on a spot plate produces a permanent brown colour. If the titration is made with uranyl nitrate, a measured quantity of a solution of sodium acetate containing acetic acid must be added in each titration; about 5 c.c. of a solution containing 100 grammes of sodium acetate and 50 c.c. of glacial acetic acid should be added in each titration. The
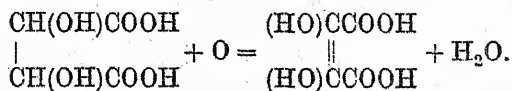
phosphate value of the uranium solution is then determined. For most purposes, it will be found convenient to express the strength of the uranium solution in terms of its equivalence to phosphorus pentoxide P_2O_5.

The uranium method of determining phosphates requires considerable care, especially as regards determining the end-point by means of the ferrocyanide indicator. The precipitation of uranium phosphate takes place somewhat slowly, with the result that a colour reaction with potassium ferrocyanide is sometimes observed before precipitation is complete, if the titration is carried out too hastily. A little practice will soon enable the operator to ascertain when the true end-point has been reached.

Determination of sodium

Sodium is a metal which forms very few insoluble salts, and consequently the precipitation of this element from solution can only be effected in a few isolated cases. It has been shown however that the sodium salt of dihydroxytartaric acid is a very sparingly soluble substance, and, since this acid may be oxidized to carbon dioxide and water by means of potassium permanganate in presence of dilute sulphuric acid, a valuable volumetric method of determining sodium has been placed in the hands of chemists. For details of the experimental procedure, the student is recommended to consult the original paper by Fenton (*Trans. Chem. Soc.*, 1898, p. 167); the principles upon which the method depends however may be briefly stated.

When tartaric acid in concentrated aqueous solution is treated with hydrogen peroxide in presence of a small quantity of a ferrous salt it undergoes oxidation to dihydroxymaleic acid. The oxidation of the tartaric acid may be represented by the equation

$$\begin{array}{c} CH(OH)COOH \\ | \\ CH(OH)COOH \end{array} + O = \begin{array}{c} (HO)CCOOH \\ \| \\ (HO)CCOOH \end{array} + H_2O.$$
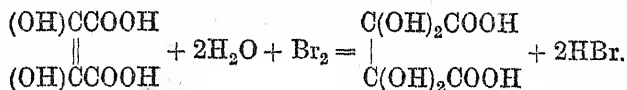
The oxidation of the tartaric acid must be effected in ice cold solution, and the isolation of the dihydroxymaleic acid is carried out by addition of fuming sulphuric acid in very small quantities

at a time. The dihydroxymaleic acid crystallizes with two molecules of water.

The next step is the conversion of the dihydroxymaleic acid into dihydroxytartaric acid by oxidation by means of bromine. The dihydroxymaleic acid is suspended in glacial acetic acid, and a slight excess of bromine added in small quantities at a time. A drop of water is occasionally added to the mixture. The reaction which takes place is represented by the equation

$$\begin{matrix} (OH)CCOOH \\ \parallel \\ (OH)CCOOH \end{matrix} + 2H_2O + Br_2 = \begin{matrix} C(OH)_2COOH \\ | \\ C(OH)_2COOH \end{matrix} + 2HBr.$$

After standing for some time, the dihydroxytartaric acid is precipitated as a heavy crystalline solid. The product is purified by washing with anhydrous ether after filtering off under pressure.

Fenton showed that when this acid is treated with potassium permanganate in presence of sulphuric acid it undergoes complete oxidation to carbon dioxide and water. It would appear that one molecule of dihydroxytartaric acid should theoretically require three atoms of oxygen for complete oxidation according to the equation

$$C_4H_6O_8 + 3O = 4CO_2 + 3H_2O.$$
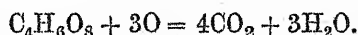
But experiment shows that the amount of oxygen required is always less than the theoretical amount. The actual quantity of oxygen required corresponds very nearly to 2·9 atoms. It is not clear to what the difference between the experimental and the theoretical values is due, although it may be due to the slow decomposition of the dihydroxytartaric acid in aqueous solution into tartronic acid and carbon dioxide. Tartronic acid is oxidized by permanganate, but with much greater slowness than dihydroxytartaric acid. The action of potassium permanganate on dihydroxytartaric acid in presence of dilute sulphuric acid is slow at first, but soon becomes much more rapid and finally becomes slow again as the end of the reaction is approached. The end-point is however quite definite.

In carrying out a determination of sodium, a suitable quantity of dihydroxytartaric acid is exactly converted into the potassium

salt by neutralization with potassium carbonate. The neutralization is effected by dissolving the acid and potassium carbonate in equivalent proportions in separate small quantities of ice cold water, and mixing the solutions. The potassium salt separates in the crystalline form. A known weight of pure sodium chloride is then taken, dissolved in the minimum quantity of water, and cooled in ice. A considerable excess of potassium dihydroxytartrate is also dissolved in the least quantity of ice cold water; the two solutions are mixed and kept at a low temperature for some time. Sodium dihydroxytartrate is precipitated by double decomposition. The precipitate is collected on a filter, washed with a small quantity of ice cold water, and dissolved in excess of dilute sulphuric acid. The resulting solution is then titrated with potassium permanganate.

It will be observed that the permanganate is standardized with reference to pure sodium chloride. Having determined the sodium value of the permanganate solution the determination of sodium in other compounds may be carried out. It is better to proceed in this way rather than to calculate the result from the fact that one molecule of dihydroxytartaric acid is oxidized by approximately 2·9 atoms of oxygen.

Determination of the hardness of waters

Natural waters are classified as hard or soft according as they require much or little soap to make a lather. The hardness of many natural waters is due to the presence of calcium or magnesium salts in solution. When a hard water is brought in contact with a soap, a substance which consists of the sodium or potassium salts of certain higher fatty acids, double decomposition takes place with the formation of a precipitate which consists of the calcium or magnesium salts of the fatty acids derived from the soap. The hardness of a water may be temporary, that is, the water is capable of being softened by boiling. Temporary hardness is due to the presence of calcium carbonate, which is held in solution by carbon dioxide in the form of a soluble bicarbonate. When the water is boiled, the carbon dioxide is expelled from solution resulting in the precipitation of the calcium carbonate. The cause of the permanent hardness of a water, that is, of the

hardness which cannot be removed by boiling, is the presence of dissolved calcium or magnesium sulphate.

A method of determining the hardness of waters was devised many years ago by Clark. This method consists in adding a standard solution of soap to a measured volume of the water with frequent shaking until a permanent lather is obtained. The first portions of the soap are used up in the precipitation of the insoluble calcium and other salts, but as soon as excess of soap has been added, a permanent lather is obtained. The soap solution is prepared by dissolving a suitable quantity of Castile soap in dilute alcohol. The solution is made of such a strength that 1 c.c. of it will precipitate exactly 1 milligramme of calcium carbonate in solution. The soap solution is standardized by weighing out one gramme of Iceland spar, dissolving this in excess of dilute hydrochloric acid, and evaporating the solution to dryness. The residue is dissolved in distilled water and diluted to one litre. Measured quantities of this solution of calcium chloride are taken, and the standard soap solution added from a burette until a permanent lather is obtained. It is important always to conduct the titrations in the same manner. The titrations are carried out in stoppered bottles in order to permit vigorous shaking. The soap solution should be added in small quantities at a time, shaking carefully between each addition. Even when the quantity of soap which is approximately required is known, the reagent should be added in the manner described. The end-point is taken as that point at which the contents of the bottle possess a permanent unbroken lather over the surface of the liquid. An experiment is also made to ascertain how much soap solution is required to produce a permanent lather with a certain volume of distilled water. This correction is applied to the result obtained in standardizing the soap solution. In determining the hardness of a water, a suitable volume of it is titrated in the manner already described. The results are usually expressed in so-called degrees of hardness or parts by weight of calcium salts or their equivalent in 70,000 parts of water. This scale corresponds to parts by weight in grains of calcium carbonate to a gallon of water. To simplify the arithmetical work it is usual to titrate 70 c.c. of the water at a time; if the soap solution is properly standardized, the degrees of hardness are obtained

directly by reference to a table.   The use of a table is necessary, since the volume of soap solution which must be added to produce a permanent lather is not strictly proportional to the amount of calcium salt present.   That is to say, if 1 c.c. of the soap solution will precipitate 1 milligramme of calcium carbonate, a solution containing n milligrammes of calcium carbonate will not require n c.c. of soap solution but a smaller volume.   This departure from direct proportionality plainly indicates that the theory of the process is much more complicated than that which has been given; nevertheless the process gives satisfactory results when properly carried out.

The process which has been described determines the total hardness, that is, the sum of the temporary and permanent hardnesses.   To determine the permanent hardness alone, a volume of the water equal to that originally taken for the determination of the total hardness is boiled for about half an hour and made up to its original volume with distilled water.   This water is then titrated with standard soap solution in the usual way.   The result gives the permanent hardness, and the difference from the total hardness gives the temporary hardness.

A preferable method of determining the hardness of water was devised by Hehner.   The temporary hardness of the water is determined by direct titration with standard sulphuric acid.   For this purpose $\frac{N}{50}$ sulphuric acid is used.   One cubic centimetre of acid of this strength will neutralize exactly one milligramme of calcium carbonate.   In carrying out a determination of the temporary hardness 100 c.c. or 70 c.c. of the water are heated nearly to boiling, a small quantity of a suitable indicator being added to the liquid.   Very good results are obtained with phenacetolin. This indicator is pink in solutions of alkaline carbonates, but golden yellow in acid solution.   The addition of the standard acid is continued until the correct colour change takes place.   The result gives the degree of temporary hardness directly in parts per 70,000 parts of water.

The permanent hardness is obtained by double decomposition with a standard solution $\left(\frac{N}{50}\right)$ of sodium carbonate.   Each cubic

centimetre of this solution will precipitate one milligramme of calcium carbonate or its equivalent in magnesium salts from solution. A measured volume of the water to be examined (70 c.c.) is taken, and a suitable known excess of standard sodium carbonate added. The solution is evaporated to dryness in a platinum dish, the soluble portion extracted with distilled water, filtered, and the filtrate titrated with standard sulphuric acid. The titration represents the amount of sodium carbonate added in excess; the difference representing the permanent hardness.

If the water contains alkali carbonate, the apparent temporary hardness as determined by titration with acid will be greater than the true value. The determination of the permanent hardness on the other hand may show more sodium carbonate than was actually added. In such a case there is no permanent hardness since the salts to which the hardness is due are decomposed by the alkali carbonate. The true temporary hardness is obtained by deducting the apparent increase in the amount of sodium carbonate added from the temporary hardness as determined by the acid titration; the result will be the true temporary hardness.

Determination of acetaldehyde

A method of determining acetaldehyde in dilute aqueous solution depends upon the reaction of this compound with a considerable excess of sodium sulphite solution resulting in the formation of the bisulphite compound according to the equation

$$CH_3CHO + Na_2SO_3 + H_2O = CH_3CH(OH)SO_3Na + NaOH.$$
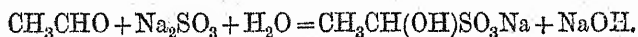
The free alkali is then determined by titration with standard sulphuric acid, phenolphthalein being employed as indicator.

The solution of sodium sulphite, which should contain about 25 per cent. of the heptahydrate, must be made just neutral to phenolphthalein before adding the dilute aldehyde. The liquid should be cooled to $0°$ C. and, after standing a short time, titrated slowly with normal sulphuric acid.

CHAPTER XI

SOME APPLICATIONS OF VOLUMETRIC METHODS

The reader who has worked through the previous chapters of this book will have become aware of the possibilities of combining two or more different volumetric processes together in order to determine the constituents of various mixtures. The availability of volumetric methods for work of this kind is very great, and it is the object of the present chapter to illustrate a few of the determinations which may be effected in this way. In many cases, alternative methods to those which are suggested are available.

(1) *Determination of oxalic and sulphuric acids when present together in the same solution*

The oxalic acid is determined by titrating a measured portion of the solution by standard potassium permanganate, the solution being as usual warmed to increase the velocity of the reaction. If the quantity of sulphuric acid present in the mixture is insufficient to prevent the precipitation of hydrated manganese dioxide, fresh dilute sulphuric acid must be added in each titration. Then in another experiment, measured portions of the solution are titrated with standard sodium hydroxide, phenolphthalein being employed as an indicator. From the titration with caustic soda we determine the quantity of soda required to neutralize both acids. Then, knowing the amount of oxalic acid which is present in a given volume of the solution from the permanganate titration, we can calculate how much sodium hydroxide has been employed in the neutralization of the oxalic acid alone. The difference between the amount of caustic soda found by experiment and that which has been employed in the neutralization of the oxalic acid is clearly equal to that which has been employed in the neutralization of the sulphuric acid, which is thus determined.

(2) *Determination of the amounts of ammonium chloride and ammonium sulphate in a mixture*

The ammonium chloride is determined by direct titration with standard silver nitrate in the usual way, potassium chromate being employed as indicator. Then another portion of the solution is boiled with a measured excess of standard sodium hydroxide until all ammonia is expelled from the solution. The amount of caustic soda remaining in excess is then determined by titration with standard acid. The difference between the amount of caustic alkali originally taken and that determined by titration is clearly equal to the amount required to decompose the ammonium chloride and sulphate in the portion of the mixture taken. In this way, the total quantity of the radical ammonium, NH_4, is determined. Knowing the amount of ammonium chloride from the titration with silver nitrate we can calculate the amount of ammonium sulphate by difference.

(3) *Determination of the amounts of sodium chloride and sodium hydroxide in a solution*

The sodium hydroxide is determined first by titration with standard nitric or sulphuric acid. Then to the neutral solution thus obtained, two drops of potassium chromate are added, and the sodium chloride determined by titration with standard silver nitrate. It is best, after determining the sodium hydroxide, to neutralize another portion of the solution exactly without the use of any indicator, and to employ the solution thus prepared to estimate the chloride, as the colour change of the chromate indicator is more easily seen in the absence of litmus or other acidimetric indicators.

(4) *Determination of the amounts of hydrochloric acid and sodium chloride in a solution*

The hydrochloric acid is determined by direct titration with caustic soda. The total chloride is then determined by titrating the neutral solution thus obtained with standard silver nitrate. As in the last example it is best to prepare an exactly neutral

B.   8

solution for determination of the chlorine without any acidimetric indicator. From the silver nitrate titration, we determine the total amount of chloride; the amount of chlorine combined with hydrogen is calculated from the titration with caustic alkali. The chlorine combined with sodium is then calculated by difference.

(5) *Determination of the amounts of ammonium chloride and sodium hydroxide in a solution*

If a solution of these two constituents be prepared, the amounts of the constituents may be determined by first titrating the sodium hydroxide with standard acid using methyl orange as indicator, and then decomposing the ammonium chloride by boiling another portion of the solution till free from ammonia, and determining by titration with standard acid the amount of sodium hydroxide remaining in excess. This procedure will clearly be successful only if the amount of sodium hydroxide in the original mixture is in excess of the amount required to decompose the ammonium salt. If the solution contains the two constituents in such proportions that the amount of sodium hydroxide is not present in sufficient quantity to effect the complete decomposition of the ammonium salt by boiling the solution, a known quantity of standard sodium hydroxide must be added to the solution before boiling. In calculating the amount of ammonium chloride in this latter case, due allowance must be made for the extra quantity of sodium hydroxide added to the mixture.

This determination might also be carried out by first determining the sodium hydroxide by neutralization with standard sulphuric or nitric acid, and then titrating the ammonium chloride with standard silver nitrate in the ordinary way. Or if the method of decomposing the ammonium salt by caustic soda be employed, the procedure might be varied by passing the evolved ammonia into a measured excess of standard acid, and then determining by titration with standard alkali the amount of acid which remains unneutralized.

A solution of a mixture of caustic soda and ammonium chloride will not keep for any length of time, as the decomposition of the ammonium salt by the action of the caustic alkali takes place even at the ordinary temperature.

Further applications

The few examples that have been described will serve to give some idea of the large variety of exercises of the kind that can be devised. Many instructive experiments may be performed by estimating the same substance in different ways. For example, the strength of a solution of ferric chloride might be determined by titration with standard potassium dichromate after reduction with stannous chloride, the excess of stannous chloride being precipitated by mercuric chloride. The same substance could also be determined by the addition of a measured excess of standard silver nitrate, the amount of silver remaining in excess being determined with standard ammonium thiocyanate.

The direct determination of substances in solution, while being the main purpose of volumetric analysis, is, however, only one of the uses of this branch of practical chemistry. Many physico-chemical determinations are effected by volumetric methods, of which mention must be made of solubilities, partition coefficients, and velocity constants of chemical reactions. We shall now give a very brief account of the methods of applying volumetric analysis to problems of this kind.

Determination of solubilities

(a) *Of solids.* The first thing to be done in determining the solubility of a solid in water is to prepare a saturated solution. This may be done in two different ways. Either a quantity of the finely powdered solid is agitated with the solvent for a considerable time, the solvent being kept at a constant temperature; or advantage is taken of the fact that the solubility of most solids is greater at high temperatures than at lower temperatures, a saturated solution at the desired temperature being prepared by cooling a solution from a higher temperature. The latter method of preparing a saturated solution usually leads to higher results than the former, since the time taken for the establishment of equilibrium between solvent and solute at the ordinary temperature is very great. For most purposes it is best to prepare the saturated solution by both methods, as the true value of the solubility must

clearly lie between the results obtained by the two methods. In all cases, it is essential to have excess of the solute in contact with the solution in order that the condition of saturation may be realized.

The strength of a saturated solution may be expressed in various ways, but the two most important are, first, to express the strength by stating that the saturated solution contains so many grammes of solute per litre, and second, by stating that a given volume of the solvent will dissolve a certain amount of solid. In both cases it is essential that the temperature be stated.

When a saturated solution has been prepared a suitable quantity of it is weighed out. Let the weight of saturated solution taken be w_1. In many cases, such a solution is much too strong to be titrated directly, it is therefore diluted to a measured extent. Aliquot portions of the diluted solution are then withdrawn and the strength determined by titration. The weight of solute in the weight of saturated solution w_1 is then calculated. Let the weight of solute thus determined be w_2. Then the quantity of water which has dissolved this weight of solute is clearly $w_1 - w_2$.

Among the various substances which are suitable for exercises in the determination of solubility may be mentioned oxalic acid, potassium dichromate, and ammonium chloride.

(b) *Of gases.* In discussing the solubility of gases in liquids, we have two main types of gas to deal with; those which obey the law of Henry, and those which do not. The former gases are the sparingly soluble ones, the solubility of which is best determined by absorptiometer methods. The latter gases are highly soluble in water and include gases such as ammonia and hydrogen chloride the properties of which lend themselves very well to solubility determination by volumetric methods. The preparation of saturated solutions of these very soluble gases is best effected by the use of a narrow U-shaped glass tube with a bulb near the bend. The bulb tube is first weighed empty. Then the bulb is about three-quarters filled with water, and the apparatus placed in a bath at a constant temperature.

The liquid in the bulb is then saturated with the gas. When the absorption of gas has been judged to be complete, the two ends of the bulb tube are sealed off. The bulb with its contents,

and of course the ends of the tube which have been removed in the sealing, are then carefully weighed. The known weight of saturated solution thus obtained is then analysed by breaking the bulb under a suitable liquid, and the resulting solution titrated. In the case of ammonia, the bulb is broken under a measured excess of standard acid, and the excess determined by titration with standard alkali, methyl orange or methyl red being employed as indicator. In the case of hydrogen chloride, the bulb may be broken under a large excess of water, and the acid determined by direct titration with caustic soda.

Determination of partition coefficients

When a substance which is soluble in each of two immiscible solvents is shaken up with them, it distributes itself between the two solvents in a particular way. If the substance possesses the same molecular weight in both solvents, then the following simple relation holds

$$\frac{c_1}{c_2} = k,$$

where c_1 and c_2 denote respectively the concentration of the substance in the first and in the second solvent, and k is a constant.

If, however, the molecular condition of the substance is different in the two solvents, the relation is a little more complicated. If the substance associates to form a complex which possesses a molecular weight which is n times as great in the second solvent to what it is in the first solvent, then we have the relation

$$\frac{c_1}{\sqrt[n]{c_2}} = k',$$

which is an immediate consequence of the law of mass action.

The constant, which is termed the ratio of distribution or the partition coefficient of the substance between the two solvents, may be determined by shaking up the solute with measured volumes of the solvents, separating the solutions thus obtained, and determining by titration the concentration of the substance

in the two solutions. Experiments are made with different total concentrations, and also with varying volumes of the two solvents. After a few determinations, it will soon be observed if the ratio of the concentrations is constant or if a more complex law is obeyed. For practice in the determination of partition coefficients, experiments may be made on the distribution of succinic acid between ether and water, and on the distribution of benzoic acid between benzene and water. In both cases, the titrations should be carried out with baryta water, phenolphthalein being used as indicator.

Determination of the velocity of chemical reactions

For an account of the theory of velocity of chemical change reference must be made to some text-book of physical chemistry. All that will be attempted here will be to illustrate the application of volumetric analysis to the determination of some substance which is taking part in a chemical change. In this connexion it may be pointed out that the usual object which the chemist has in view in determining velocity constants is to determine the so-called "order" of the reaction. While it is true that the order of a reaction agrees in many cases with the actual number of molecules which are represented as taking part in the reaction by the chemical equation, it is an undoubted fact that in a large number of cases the order of the reaction is found to be less than the number of molecules which the chemical equation represents. While this does not demand an immediate revision of the theory of the subject, it certainly suggests the necessity of a less stringent application of the order of a reaction as determined by measurement of the reaction velocity to the estimation of the number of molecules which take part in a chemical reaction.

All experiments on velocity of reaction must be performed under conditions which maintain the reacting substances at a constant temperature, since the temperature coefficient of velocity is very great. In many cases a rise of 10° C. doubles or trebles the reaction velocity.

A reaction which may be studied with success by an acidimetric method is the decomposition of dibromosuccinic acid into

bromomaleic acid and hydrobromic acid. This reaction takes place in aqueous solution according to the equation

$$C_2H_2Br_2(COOH)_2 = C_2HBr(COOH)_2 + HBr,$$

and proceeds at a velocity suitable for experimental determination at a temperature of 100° C. It is clear that the solution becomes more and more acid as the reaction proceeds; the velocity of the reaction may therefore be measured by withdrawing portions of the solution at definite intervals of time and determining the acidity by titration with standard alkali.

It will be found that the rate of disappearance of the dibromo-succinic acid is at every instant proportional to the amount of this substance present in the solution or as represented by the differential equation

$$-\frac{dC}{dt} = kC,$$

where C denotes the concentration of the dibromosuccinic acid, t the time, and k the velocity constant of the reaction. The reaction is therefore a unimolecular one or as it is frequently termed a reaction of the first order. In this case, the order of the reaction is a true measure of the number of molecules taking part in the reaction.

The few examples which have been quoted will serve to convey some idea of the wide applications of volumetric work. For more detailed application we must refer the reader to special treatises.

CHAPTER XII

SOME EXAMPLES OF VOLUMETRIC DETERMINATIONS

The following examples of volumetric determinations have been given chiefly with the view of illustrating some of the inter-relationships between different processes, and also of demonstrating the order of magnitude of the errors involved. The determinations were all carried out with ordinary measuring vessels which had not been calibrated: if accurately calibrated vessels had been employed, the results would, of course, have been considerably more accurate.

(1) *Determination of the strength of a solution of potassium dichromate by means of ferrous ammonium sulphate*

A quantity of the double iron salt was purified by recrystallization from hot water, a few drops of dilute sulphuric acid being added to prevent the formation of basic salt. After drying the salt by pressing between filter paper, a standard solution was prepared, and aliquot portions of this solution titrated by means of the given solution of potassium dichromate, potassium ferricyanide being as usual employed as an external indicator. The following results were obtained:

Weight of ferrous ammonium sulphate taken = 5·950 grammes.

This salt was dissolved in water with the addition of dilute sulphuric acid, and the solution diluted to 200 c.c.

Two titrations were made with 20 c.c. of the iron solution; in both cases the volume of potassium dichromate required was 15·0 c.c.

From this it follows that the volume of the dichromate solution which would be required for the complete oxidation of

the 5·950 grms. of the double iron salt (corresponding to 0·850 grm. of iron) = 150 c.c.

The strength of the solution of potassium dichromate is calculated from the equation

$$\frac{150x}{0\cdot850} = \frac{294}{6 \times 56},$$

from which $x = 0\cdot00496$ grm. $K_2Cr_2O_7$ per c.c.

(2) *Determination of the amount of arsenious oxide in a solution of sodium arsenite by means of pure arsenious oxide*

This determination was carried out by carefully purifying some arsenious oxide by sublimation, preparing a standard solution of sodium arsenite from this, and titrating first the standard solution of alkali arsenite and then the unknown solution of sodium arsenite with a solution of iodine of unknown strength. The titrations were carried out in the usual manner with the addition of excess of sodium bicarbonate to the arsenic solutions, and the end-point determined with the aid of starch. The following results were obtained:

Weight of resublimed arsenious oxide taken = 0·898 grm.

This solid was dissolved in a solution of sodium carbonate containing 4 grms. of the solid carbonate, and the liquid diluted to 200 c.c.

The standard solution of sodium arsenite prepared as above described was titrated with a solution of iodine of unknown strength. Two titrations with 20 c.c. of the arsenic solution required in each case 29·95 c.c. of iodine.

The given solution of sodium arsenite was next titrated with the same iodine solution.

Two titrations with 20 c.c. of the solution in each case required 32·1 c.c. of iodine.

The calculation of the strength of the given solution of sodium arsenite is made from the equation:

$$\frac{\text{Concentration of As}_2O_3 \text{ in the given solution}}{\text{Concentration of As}_2O_3 \text{ in the standard solution}} = \frac{32\cdot1}{29\cdot95}.$$

The standard solution contained 0·00449 grm. of As_2O_3 per c.c.

Therefore the weight of As_2O_3 in each c.c. of the given solution of sodium arsenite

$$= \frac{32 \cdot 1 \times 0 \cdot 00449}{29 \cdot 95} \text{ grm.}$$

$$= 0 \cdot 00481 \text{ grm.}$$

(3) *Determination of the strength of a solution of sodium thio-sulphate by means of a solution of sodium arsenite of known strength*

The reactions of iodine towards arsenious oxide and towards sodium thiosulphate being known, it is a simple matter to determine the strength of a solution of this latter substance by titration with a solution of iodine, the arsenic value of which is known. Accordingly the given solution of sodium thiosulphate was titrated with the same solution of iodine which had been used for titrating the arsenic solution in (2). Two titrations were made, 20 c.c. of the thiosulphate solution required 32·8 c.c. of iodine in each case.

In calculating the strength of the solution of sodium thiosulphate, it is to be borne in mind that 127 parts by weight of iodine react with 158 parts by weight of sodium thiosulphate (anhydrous) with formation of sodium iodide and tetrathionate, and that 4×127 parts by weight of iodine are capable of oxidizing 198 parts by weight of arsenious oxide. Consequently the equivalent weights of sodium thiosulphate and arsenious oxide are in the ratio of 4×158 to 198.

In the previous determination it was found that 20 c.c. of the solution of sodium arsenite containing 0·00481 grm. of As_2O_3 per c.c. required 32·1 c.c. of iodine solution. The volume of sodium thiosulphate solution equivalent to that volume of iodine is clearly $\dfrac{20 \times 32 \cdot 1}{32 \cdot 8}$ c.c. or 19·57 c.c. Denoting by y the weight of $Na_2S_2O_3$ per c.c., we determine y by solving the equation

$$\frac{19 \cdot 57 y}{20 \times 0 \cdot 00481} = \frac{4 \times 158}{198},$$

from which $y = 0 \cdot 0157$ grm. of $Na_2S_2O_3$ per c.c.

(4) *Determination of the strength of a solution of potassium dichromate iodometrically*

The solution of potassium dichromate, the strength of which was determined by means of recrystallized ferrous ammonium sulphate in (1), was treated with excess of potassium iodide in presence of a little dilute sulphuric acid and the liberated iodine titrated by means of standard sodium thiosulphate. The sodium thiosulphate solution was that standardized in (3). As a mean of several concordant titrations, it was found that 20 c.c. of the dichromate solution required 20·2 c.c. of thiosulphate. The strength of the solution of potassium dichromate x in grms. per c.c. was therefore obtained by solving the equation

$$\frac{20x}{20·2 \times 0·0157} = \frac{294}{6 \times 158},$$

from which $x = 0·00492$ grm. of $K_2Cr_2O_7$ per c.c.

Comparing this result with that obtained in (1) in which the strength of the potassium dichromate solution was determined by means of recrystallized ferrous ammonium sulphate, the difference approximates to one per cent. Taking into account the fact that the solution of sodium thiosulphate was standardized by a somewhat indirect method, the agreement between the iron method and the iodometric method of determining the strength of the dichromate solution must be regarded as satisfactory.

(5) *An experiment to illustrate the relative magnitude of the errors involved by differences in the relative volumes of the measuring vessels*

In this experiment the strength of a solution of iodine was determined by means of the standard sodium thiosulphate in (3) placing first the iodine in the pipette and the thiosulphate in the burette, and secondly with the thiosulphate in the pipette and the iodine in the burette. The relative volumes of the pipette and of measured volumes from the burette were then determined by weighing the volumes of water delivered from these vessels. The following were the results obtained:

20 c.c. of iodine measured out by the pipette required as a mean result 20·3 c.c. of thiosulphate.

From this determination, the strength of the iodine equals 12·8 grms. per litre.

20 c.c. of sodium thiosulphate measured out by the pipette required 20·0 c.c. of iodine from the burette.

This determination gives the strength of the iodine solution as 12·6 grms. of iodine per litre. It is clear that the agreement between the two determinations is of the order of a difference of one-and-one-half per cent. This difference cannot possibly be due to any difficulty in carrying out the experiments, as the reaction between iodine and sodium thiosulphate is one of the most accurate reactions with which we have to deal, and the end-point is particularly easy to determine. It was therefore of interest to compare the relative volumes of the 20 c.c. pipette and of 20 c.c. of the burette.

It was found as a mean of several concordant measurements that the weight of water delivered from the pipette was 20·12 grms., while the weight of water delivered by running out 20 c.c. of water from the burette (taken between different points) was 19·92 grms. This difference was two parts in 200, or in other words the volume of the pipette was approximately one per cent. greater than that of an apparently equal volume of the burette.

(6) *Standardization of a solution of hydrochloric acid by means of sodium*

The hydrochloric acid was prepared so as to approximate to normal strength. A weighed piece of freshly cut sodium was dissolved in alcohol, and after the metal had been completely dissolved, excess of water was added to the solution. The solution thus obtained was next titrated with the hydrochloric acid, methyl red being used as indicator.

In the first experiment 0·454 grm. of sodium required 19·7 c.c. of hydrochloric acid.

Denoting by x the weight of hydrogen chloride in grms. per c.c. we obtain x by solution of the equation

$$\frac{19\cdot7x}{0\cdot454} = \frac{36\cdot5}{23},$$

from which $x = 0\cdot0366$ grm. HCl per c.c.

In the second experiment 0·659 grm. of sodium required 28·6 c.c. of hydrochloric acid.   From which we have

$$\frac{28·6x}{0·659} = \frac{36·5}{23},$$

or $x = 0·0366$ grm. HCl per c.c.

The agreement between the two experiments is perfect.   It is clear that the hydrochloric acid is very nearly of normal strength, the normal solution containing 0·0365 grm. HCl per c.c.

(7)   Determination of the strength of a solution of potassium hydroxide by means of standard hydrochloric acid

The hydrochloric acid the strength of which was determined by sodium in (6) was employed for titrating the solution of potash, methyl orange being employed as indicator.

In two experiments it was found that 20 c.c. of KOH required 19·5 c.c. of standard HCl.

Let y denote the strength of the solution of potash in grms. per c.c.   Then we have

$$\frac{20y}{19·5 \times 0·0366} = \frac{56}{36·5},$$

from which $y = 0·0547$ grm. per c.c.

(8)   Determination of the strength of a solution of potassium permanganate by means of a standard solution of potassium dichromate

A solution of ferrous sulphate containing sulphuric acid was prepared, and aliquot portions of this solution were titrated in turn with the standard solution of potassium dichromate in (1) and with the solution of potassium permanganate.   As a result of several concordant experiments it was found that 25 c.c. of the iron solution required 24·6 c.c. of potassium dichromate and 24·3 c.c. of potassium permanganate.

It is clear that $24·6 \times 0·00496$ grm. of potassium dichromate is equivalent in oxidizing power to $24·3x$ grms. of potassium permanganate, where x denotes the weight of potassium permanganate in grms. per c.c.   It is also an experimental fact that 316 parts by

weight of potassium permanganate are equivalent to 490 parts by weight of potassium dichromate in oxidizing power. The value of x is therefore determined from the equation

$$\frac{24 \cdot 3x}{24 \cdot 6 \times 0 \cdot 00496} = \frac{316}{490},$$

from which $x = 0 \cdot 00324$ grm. $KMnO_4$ per c.c.

(9) Determination of the strength of a solution of oxalic acid by means of standard potash

Since oxalic acid is a weak acid and potassium hydroxide a strong base, phenolphthalein was employed as the indicator. The solution of potash in (7) was diluted to one-tenth of its original strength, making the strength $0 \cdot 00547$ grm. per c.c. The mean result of two concordant titrations was that 20 c.c. of the oxalic acid required the addition of $23 \cdot 35$ c.c. of potash,

$$\frac{20x}{23 \cdot 35 \times 0 \cdot 00547} = \frac{45}{56},$$

from which $x = 0 \cdot 00513$ grm. of $\begin{matrix} COOH \\ | \\ COOH \end{matrix}$ per c.c.

(10) Determination of the strength of the solution of the oxalic acid in (9) by means of standard potassium permanganate

The permanganate was that standardized in (8). Aliquot portions of the solution of oxalic acid were taken, a small quantity of sulphuric acid added to each, and each solution was heated before running in the permanganate. In the first experiment, the volume of permanganate required for complete oxidation of 20 c.c. of the solution of oxalic acid was $22 \cdot 5$ c.c., in the second experiment it was $22 \cdot 4$ c.c. From these results we have

$$\frac{20x}{22 \cdot 45 \times 0 \cdot 00324} = \frac{5 \times 90}{316},$$

from which $x = 0 \cdot 00517$ grm. of $\begin{matrix} COOH \\ | \\ COOH \end{matrix}$ per c.c.

The difference between the two results in (9) and (10) is less

than one per cent., and this order of accuracy is as good as can be expected when it is borne in mind that both the potassium hydroxide and permanganate were standardized in a somewhat indirect manner.

(11) *Determination of the strength of a solution of silver nitrate by means of standard hydrochloric acid*

The hydrochloric acid prepared in (6) was diluted to one-tenth of its original strength as the solution of silver nitrate was prepared so as to be of approximately deci-normal strength. Aliquot portions of the solution of hydrochloric acid were measured out, and excess of calcium carbonate added to each. The contents of each flask were then titrated by means of silver nitrate, potassium chromate being employed as an indicator. As the mean of two concordant titrations it was found that 20 c.c. of the diluted hydrochloric acid required the addition of 20·25 c.c. of silver nitrate for complete precipitations. The weight x in grms. of silver nitrate in each c.c. of the given solution is therefore determined by the equation

$$\frac{20 \cdot 25x}{20 \times 0 \cdot 00366} = \frac{170}{36 \cdot 5},$$

from which $x = 0 \cdot 0168$ grm. $AgNO_3$ per c.c.

(12) *Determination of the amounts of hydrochloric acid and oxalic acid in a solution of the mixture*

This mixture was prepared by adding 500 c.c. of the solution of hydrochloric acid in (6) to 2000 c.c. of the solution of oxalic acid in (9) and (10). Determinations of the specific gravities of the constituent solutions and also of the resulting mixture showed that the total volume was very nearly 2500 c.c.; the difference between the observed and calculated values of the specific gravity of the mixture being negligible. The calculated values of the two acids in the mixture were therefore 7·32 grms. of hydrochloric acid and 4·12 grms. of oxalic acid (anhydrous) per litre.

This determination was carried out by titrating the hydrochloric acid by means of standard silver nitrate after addition of excess of calcium carbonate to the solution, potassium chromate

being employed as an indicator. Then a further quantity of the mixture was measured out, and the mixed acids titrated by means of standard potassium hydroxide using phenolphthalein as indicator. The first experiment determined the weight of hydrochloric acid in a given volume of the mixture*. From the quantity of potash which is required to neutralize a definite volume of the mixed acids, it is possible to determine how much of the potash has been employed in the neutralization of the hydrochloric acid alone. The difference between the amount of potash which has been used up in the neutralization of the hydrochloric acid and that which has been required for the neutralization of the total acid is clearly equal to the amount which has been employed in the neutralization of the oxalic acid.

The standard solution of potash was diluted to one-fifth of its original strength making its new strength 0·0109 grm. per c.c.

It was found that 20 c.c. of the mixed acids required as a mean result 30·2 c.c. of potash and also that 20 c.c. of the mixture required 40·35 c.c. of standard silver nitrate.

From the result of the silver nitrate experiment it was found that the solution contained 7·29 grms. of hydrochloric acid per litre.

The weight of potassium hydroxide required to neutralize 7·29 grms. of hydrogen chloride is clearly equal to $\dfrac{56 \times 7\cdot29}{36\cdot5}$ grms. or 11·2 grms.

The weight of potash required to neutralize one litre of the mixture $= 50 \times 30\cdot2 \times 0\cdot0109$ grms. or 16·5 grms.

Hence the weight of potash required for the neutralization of the oxalic acid alone equals $(16\cdot5 - 11\cdot2)$ grms. or 5·3 grms.

Since 56 parts by weight of potassium hydroxide neutralize 45 parts by weight of oxalic acid (anhydrous), it is clear that the weight of oxalic acid neutralized by 5·3 grms. of potash $= \dfrac{45 \times 5\cdot3}{56}$ grms. or 4·26 grms.

The given solution contains therefore 7·29 grms. of hydrochloric acid and 4·26 grms. of oxalic acid (anhydrous) per litre.

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* It is necessary to neutralize with calcium carbonate in order to precipitate the very sparingly soluble calcium oxalate, and thereby prevent the precipitation of silver oxalate.

Calculation of the weights of the two acids from a single titration with potash

The total weight of the mixed acids in one litre of the solution equals 11·44 grms.

Let x denote the weight of oxalic acid and y the weight of hydrochloric acid per litre, then

$$x + y = 11\text{·}44 \dots\dots\dots\dots\dots\dots(1),$$

$$\frac{56x}{45} + \frac{56y}{36\text{·}5} = 30\text{·}2 \times 0\text{·}0109 \times 50 \dots\dots\dots\dots(2).$$

Solution of these two simultaneous equations gave the following results:

$x = 3\text{·}62$ grms. of oxalic acid (anhydrous) per litre and

$y = 7\text{·}82$ grms. of hydrochloric acid per litre.

This example is sufficient to illustrate the limitations of the accuracy of the general method of determining the amounts of two constituents of a mixture when the total weight is known from the difference between the equivalent weights, a small error in the experimental work giving rise to a considerable error in the final result especially when the two constituents are not present in roughly equal proportions.

CHAPTER XIII

SIMPLE GRAVIMETRIC DETERMINATIONS

The fundamental principle involved in gravimetric analysis is the conversion of the substance which is to be determined into some derivative of definitely known composition, and capable therefore of being weighed accurately. It is essential that the substance which is to be prepared for this purpose shall be easily separated in a condition of great purity. Very sparingly soluble substances are most commonly employed for this purpose, because of the ease with which they may be separated and purified by filtration and washing. For example, the percentage of chlorine in a soluble chloride can be determined by dissolving a known quantity of the substance in water acidified with nitric acid, adding excess of a solution of silver nitrate, when double decomposition takes place resulting in the formation of silver chloride, which is subsequently collected and weighed. In other cases separation may be effected by taking advantage of some property other than that of insolubility. For instance, the determination of the water of crystallization in a hydrated salt may sometimes be effected by raising the salt to a suitable temperature to dissociate the hydrate and thereby convert the substance into the anhydrous salt.

Manipulation and sources of error

Using an ordinary analytical balance it is a simple matter to weigh accurately with the rider to half a milligramme. If therefore the weight of substance is of the order of 0·5 gramme and the error of the weighing is ± 0·0005 gramme the experimental error is one part in one thousand. Greater accuracy than this is not easy to secure under ordinary conditions. In commencing the analysis a quantity of the original substance should be taken which may be expected to produce a yield of substance for the final weighing which is likely

to involve an error of this order of magnitude. Beginners usually err on the side of dealing with too large quantities of substance. It is true that the larger the quantity of substance which is weighed the smaller the error will be; but a large quantity of substance involves a bulky precipitate which is difficult to filter and wash, and the errors due to the weighing of impure material are usually considerable.

It is absolutely essential that great attention should be paid to care in manipulation. Success in mastering the details can only be learnt by practice in the laboratory, but a few general directions may be given to assist the beginner.

A knowledge of the specific properties of the substance which is to be prepared for carrying out the determination is of importance. Most sparingly soluble substances are at their maximum solubility when they are just freshly precipitated from solution, but usually become more insoluble by being kept for some little time before being filtered. The properties of a precipitate may be modified very considerably by varying the conditions under which it is produced: the most important of which are the temperature and concentration of the solutions. Errors may arise from two sources. In the first place, the slight solubility of a precipitate may result in a low yield of the substance for the final weighing. Secondly, many precipitates possess the property of occluding or adsorbing other substances from solution which are not completely removed even with prolonged washing. Care must therefore be taken to reduce these two opposite errors to a minimum.

Unless the properties of the substance determine otherwise, it is desirable to carry out precipitation in a boiling solution in order to obtain the precipitate in as granular a condition as possible to facilitate the operations of filtering and washing. When precipitation is effected at the boiling point, it is usually safer to filter the liquid shortly afterwards than when the precipitate is produced at the ordinary temperature, because heat accelerates the completion of the process. In some cases, however, the solubility of the precipitate is increased by rise of temperature to an extent which necessitates carrying out the process at the ordinary temperature.

Filtration may be effected with the aid of filter papers, or by the use of specially prepared asbestos which is placed in Gooch crucibles. Filter papers can be used for any precipitate, and usually yield the best results in the hands of beginners. On the other hand, many

precipitates can be filtered with much greater rapidity with the aid of a pump in Gooch crucibles provided with asbestos, and students are recommended to carry out some of their determinations in that way. If filter papers are used, they must be of known ash content, and care must be taken that they fit accurately into the funnel. The filter paper must on no account project beyond the funnel. If Gooch's method of filtration is employed, attention must be paid to the quantity of asbestos which is used. The amount of asbestos should be sufficient to make the holes just perceptible when the crucible is viewed against a good light. As the subsequent operations of washing, drying, and weighing the precipitate are effected in the Gooch crucible, the crucible with its asbestos must be heated and allowed to cool in a desiccator and then carefully weighed before carrying out the determination.

It is perhaps in the operation of washing that the greatest attention to detail is necessary in order to secure the maximum accuracy. The best method of procedure is that known as washing by decantation. Precipitation having been effected, the precipitate is allowed to settle, and the clear liquid is then carefully poured down a glass rod into a filter or Gooch crucible, care being taken to keep back as much of the precipitate as possible. Water is then added to the precipitate which is again allowed to settle, and the clear liquid is once more poured down the glass rod into the filter. These operations are repeated three or four times before the main quantity of the precipitate is transferred to the filter. The washing is then completed on the filter. The object is to do as much of the washing as possible in the beaker rather than on the filter paper or Gooch crucible. The washing of the precipitate on the filter must be continued until a portion of the filtrate shows by qualitative tests the absence of salts which are being washed from the precipitate.

It is desirable whenever possible to wash a precipitate with hot water, but this is not possible in all cases. Sometimes it is necessary to employ other reagents. For example, in the determination of magnesium as the ammonio phosphate, it is not possible to wash the precipitate with pure water, because the substance undergoes slow hydrolysis and passes into solution to an extent sufficient to cause serious error. Hydrolysis in this particular case may however be checked by washing the precipitate with dilute ammonia instead of with pure water, and satisfactory results are readily obtained.

When the process of washing has been completed it is necessary to prepare the precipitate for the final weighing. In most cases this is done by ignition at a suitable temperature, but if the substance is of such a nature as to preclude this mode of treatment, the precipitate must be dried at a temperature at which it does not suffer decomposition.

Assuming that the precipitate is to be ignited, a small crucible with its lid are heated to redness, allowed to cool in a desiccator and then carefully weighed. If the precipitate is of such a nature as to be liable to suffer reduction from the products of combustion of the filter paper, the paper and its contents must first be dried with the aid of heat, the precipitate carefully removed from the paper, which is ignited apart from it, and the precipitate and the filter ash finally ignited together in the crucible. The crucible and lid with their contents are then cooled in the desiccator and weighed. The ignition and weighing must be repeated until the weight is constant. If there is no danger of reducing the precipitate by contact with the gases from the burning filter paper, the precipitate and filter paper may be ignited together; and indeed previous drying is unnecessary. The moist precipitate and filter paper may be placed directly in the crucible, a very small flame being used to drive off moisture, care being taken to avoid any risk of loss of material. The flame is then gradually raised, and the ignition completed at a red heat.

If the determination is carried out in a Gooch crucible, the precipitate after thorough washing is drained with the aid of the filter pump, and the crucible with its contents placed inside an ordinary larger crucible, and either ignited at a red heat or simply heated to some lower temperature depending upon the nature of the precipitate. If the substance is liable to be reduced at a high temperature, care must be taken to avoid the access of reducing gases from the flame. In either case the crucible and its contents must be brought to constant weight before the result of the analysis is calculated.

In a few cases determinations are carried out with the aid of tared filters, when the nature of the precipitate is such as to render Gooch's method of filtration unsuitable, and the substance cannot be heated to a temperature above 100° C. without decomposition. The filter paper may be dried at 100° C. or by exposure over con-

centrated sulphuric acid in a vacuum desiccator at the ordinary temperature, placed in a suitable weighing tube, and then carefully weighed. The operations of filtering and washing are then carried out in the ordinary way, and the filter paper and its contents dried in exactly the same way at the conclusion of the experiment as previously. The weight of the precipitate is then simply determined by difference.

Determination of iron

Iron is weighed as ferric oxide, Fe_2O_3, which is obtained by precipitation as ferric hydroxide by adding ammonia in slight excess to a solution of a ferric salt. If a ferrous salt is to be analysed, the iron must first be completely oxidized to the ferric condition before precipitation with ammonia.

For practice in the estimation, weigh out about one gramme of recrystallized ferrous ammonium sulphate, $FeSO_4(NH_4)_2SO_46H_2O$, dissolve the salt in about 100 to 150 c.c. of distilled water. Then add a little nitric acid and boil the liquid for a short time to oxidize the solution to ferric sulphate. Add a slight excess of a solution of ammonia to the hot liquid, and allow the precipitate to settle. Then filter and wash the precipitate by decantation with hot water, and continue the washing until a test portion of the filtrate shows the absence of ammonium sulphate, as indicated by adding a little hydrochloric acid and barium chloride. Finally ignite the precipitate to constant weight over a Bunsen burner.

If w_1 grms. of the double iron salt yield w_2 grms. of ferric oxide, the weight of iron is equal to $\dfrac{55 \cdot 8 \times 2w_2}{55 \cdot 8 \times 2 + 16 \times 3}$ from which the percentage of iron can be calculated.

Ferric hydroxide is a bulky reddish-brown precipitate, insoluble in water and in dilute ammoniacal liquids, but readily soluble in acids. When dried it shrinks greatly in bulk, but a high temperature is necessary to drive off all the water. The anhydrous ferric oxide which is obtained by ignition is fairly stable at high temperatures and not easily reduced. Some chemists consider that there is a risk of partial conversion into the magnetic oxide, Fe_3O_4, at very high temperatures. The ignited oxide is very difficult to dissolve in acids.

This method of analysis can be used only in the absence of other substances which are precipitated by ammonia. If manganese is present, the usual process of separating iron by adding ammonium

chloride before ammonia, which is quite satisfactory in qualitative analysis, cannot be used because the separation is incomplete. In quantitative work it is necessary to precipitate the iron as basic ferric acetate, which is subsequently ignited and weighed as ferric oxide.

Determination of the sulphate radical in soluble sulphates

Sulphuric acid and soluble sulphates, and indeed sulphur in any form, are determined by conversion into barium sulphate, $BaSO_4$, which is afterwards weighed. Although the process would seem to be a very simple one, great care is necessary in order to obtain accurate results. The reason of this is that barium sulphate is very liable to adsorb certain salts from solution particularly when it is just precipitated, and it is frequently very difficult to remove these salts completely by washing.

The quantity of material to be taken for the analysis must be judged from the atomic weight of the metal and the amount of water of hydration, if any, in the salt. For example, in the case of copper sulphate, the formula weight of which is 249·6 as the salt crystallizes with five molecules of water, a suitable quantity would be about 0·5 gramme. The calculated weight of barium sulphate from such a quantity of copper sulphate would be of that order of magnitude. On the other hand, a smaller quantity of potassium sulphate might with advantage be used, as this latter salt is anhydrous and the atomic weight of potassium is 39·1, as against that of copper which is 63·6.

The requisite quantity of the soluble sulphate should be dissolved in about 100 c.c. of distilled water, and acidified with a small quantity of hydrochloric acid. The liquid is now heated to the boiling point, and a solution of barium chloride added very slowly, preferably drop by drop, to the contents of the beaker. The precipitate of barium sulphate is then allowed to settle, and another drop of barium chloride added in order to ascertain whether precipitation is complete. If the precipitation is not complete more barium chloride must be added until this is the case. The beaker and its contents should be heated for some little time in a water bath before filtration. The precipitate is then filtered and washed by decantation, using hot water; and filter paper will be found to give better results than a Gooch crucible.

The washing must be continued until a test portion of the filtrate shows the absence of barium chloride as indicated by adding a few drops of dilute sulphuric acid. The precipitate should be ignited at a bright red heat, and it is quite safe to ignite along with the filter paper. Formerly it was considered that there was danger of partial reduction of the barium sulphate to barium sulphide, but if the ignition is conducted with free access of air to the precipitate, the risk is practically *nil*. The crucible is then allowed to cool in a desiccator and weighed. The ignition must be repeated until the weight is constant.

The weight of barium sulphate multiplied by $\dfrac{96}{137\cdot4 + 96}$ represents the weight of the sulphate radical, and the calculation of its percentage from the weight of substance originally taken can easily be made.

Barium sulphate is a fine white powder as usually obtained by precipitation of a soluble sulphate with a solution of a barium salt. It is practically insoluble in water, but its solubility may be increased sufficiently to introduce serious error into an analysis if the solution contains considerable quantities of free acid. Small quantities of hydrochloric acid are without serious influence upon its solubility. The properties of the solid depend to a considerable extent upon the concentration of the solution from which it is deposited. When freshly precipitated, the particles are smaller than when the solid has been kept in contact with the solution for some time. Heat also accelerates the growth of the crystalline particles. It has been shown that the solubility of barium sulphate depends upon the size of the particles, the smaller particles having a greater solubility than the larger ones.

Determination of copper

Copper may be determined by several gravimetric methods, the best of which is doubtless the electrolytic determination as the metal. Of the non-electrolytic methods, that as cuprous thiocyanate, CuCNS, is capable of yielding excellent results.

For practice in the method weigh out about 0·8 gramme of recrystallized cupric sulphate, dissolve the salt in about 100 c.c. of distilled water. Then add a small quantity of a concentrated solution of sulphur dioxide, followed by a slight excess of a solution of

ammonium thiocyanate. Cuprous thiocyanate is precipitated as a white or faintly pink granular solid. The precipitation should be carried out at the ordinary temperature, and the liquid should be thoroughly stirred. The liquid should be kept for some time at the ordinary temperature before filtering to ensure complete precipitation. The precipitate may be filtered with advantage in a Gooch crucible, washed thoroughly with cold water until the filtrate shows only a faint pink coloration when tested with ferric chloride, and then the washing completed with dilute (20 per cent.) alcohol. The crucible with its contents are dried in an air bath at a temperature of about 120° C., and then cooled in a desiccator and weighed. The process is then repeated until the weight is constant.

The ratio $\dfrac{63\cdot6}{63\cdot6 + 58}$ multiplied by the weight of cuprous thiocyanate will give the required weight of copper.

Cuprous thiocyanate is practically insoluble in cold water, but its solubility in hot water is appreciable. Nitric acid and other oxidizing agents interfere with the precipitation of this substance. It is quite stable at temperatures up to about 150° C., but at higher temperatures it decomposes.

The determination of chlorine in a soluble chloride and of silver in an alloy

Soluble chlorides are determined by conversion into silver chloride, AgCl, which is practically insoluble in water, and similarly silver in alloys which contain that metal is determined by dissolving the alloy in nitric acid and precipitating the metal as silver chloride by adding hydrochloric acid. In both cases the constituent is determined by weighing the resulting silver chloride.

As an example of the former process, we may consider the determination of the percentage of chlorine in sodium chloride. About 0·3 to 0·4 gramme of the salt is dissolved in about 100 c.c. of distilled water acidified with nitric acid, and the resulting solution heated to the boiling point. A slight excess of a solution of silver nitrate is then added drop by drop until the formation of the precipitate ceases. The precipitate is then allowed to settle and then another drop of silver nitrate is added to ascertain if precipitation is complete. The liquid is then filtered and washed by decantation, first with water containing a very little nitric acid,

and afterwards with pure water. A portion of the filtrate must be tested from time to time with hydrochloric acid, and the washing continued until no reaction is obtained. Silver chloride can be filtered and washed with ease in a Gooch crucible. The crucible and its contents should be dried at a temperature of about 150° C. until the weight is constant. The weight of silver chloride multiplied by $\dfrac{35 \cdot 5}{143 \cdot 5}$ will give the weight of chlorine in the weight of sodium chloride taken for analysis.

We may now consider the gravimetric process of determining the percentage of silver in a silver alloy. A suitable weight of the alloy, say 0·4 gramme, is weighed out and dissolved in dilute nitric acid (about two parts of water to one of nitric acid). The solution of the metal may be effected very conveniently in a small flask inclined at an angle in order to allow the spray caused by the evolution of gases to impinge against the vessel and thus avoid loss. The liquid should be thoroughly boiled during the solution of the alloy. The contents of the flask are then rinsed into a beaker and diluted with water. A slight excess of hydrochloric acid is then added to precipitate the silver as silver chloride. The filtration and washing of the precipitate are carried out precisely as in the previous experiment, except that qualitative tests are applied to ascertain the removal of hydrochloric acid instead of silver nitrate.

As produced by double decomposition between silver nitrate and a soluble chloride, silver chloride is a white precipitate which readily collects into a curdy flocculent condition. The filtration of this substance presents very little difficulty. It is very nearly insoluble in water. Under certain conditions it is liable to pass into solution to some extent in a colloidal condition, but the presence of a little nitric acid prevents this. Silver chloride is sensitive to light, partial reduction taking place and the solid assuming a violet colour. In accurate determinations it is necessary to carry out all the manipulations in the absence of actinic light. Silver chloride can be dissolved by various substances of which mention may be made of aqueous solutions of ammonia, of potassium cyanide, and of sodium thiosulphate; in each case complex salts are formed. It is stable as regards the action of heat up to a temperature of 451° C. when it melts. Care must therefore be taken not to overheat silver chloride in a Gooch crucible as it will melt and

run through the holes. Silver chloride is easily reduced to the metal
when heated in contact with reducing gases. If therefore the deter-
mination is carried out with a filter paper, it is essential to remove
the precipitate as completely as possible from the paper before the
latter is ignited. The ash of the filter paper should be moistened
with a drop of nitric acid and a couple of drops of concentrated
hydrochloric acid and again heated to reconvert any reduced silver
into the chloride. It is obvious that the properties of the substance
are such as to recommend the use of the Gooch crucible.

Silver bromide and iodide are similar in properties to the
chloride, and are therefore used for the determination of bromine
and iodine, and the method is identical.

Determination of calcium

Calcium is usually determined by precipitation as the oxalate
which is subsequently converted into the carbonate or the oxide.
It may be weighed either as carbonate or as oxide.

In order to determine the percentage of calcium in Iceland spar,
weigh out about 0·5 gramme of the solid and dissolve it in a little
dilute hydrochloric acid, taking care to avoid loss by effervescence.
The liquid is then diluted with water and boiled. A little ammonium
chloride and a slight excess of ammonia are then added. A slight
excess of ammonium oxalate is then added to precipitate the
calcium. The liquid should be heated in a water bath for some time
before filtering. The filtration and washing may be effected in a
Gooch crucible or a filter paper may be used; the latter is perhaps
preferable. The washing must be continued until a test portion of
the filtrate shows the absence of chloride.

If the calcium is to be weighed as the carbonate $CaCO_3$, the
oxalate must be ignited at a temperature not exceeding 500° C.,
which may be judged as approximating to visible redness. Under
such conditions the oxalate loses carbon monoxide only, $CaC_2O_4 =
CaCO_3 + CO$. If a Gooch crucible is used, the process will not
present any difficulty; but if a filter paper is used, there may be
a difficulty in burning the paper completely without danger of
heating the precipitate too strongly. In such a case it is perhaps
the simplest plan to ignite the precipitate apart from the filter
paper. In any case, if the precipitate is heated too strongly,
resulting in the transformation of some of the calcium carbonate

into the oxide, it is only necessary to add a little solid ammonium carbonate to the contents of the crucible, when the oxide will be completely reconverted into carbonate. The crucible is then cooled in a desiccator and weighed. The ignition and weighing must be repeated until the weight is constant.

If the calcium is to be weighed as oxide, a high temperature is necessary to dissociate the carbonate completely. The moist precipitate and filter paper should be placed in a crucible and heated with a very small flame to drive off all moisture. The temperature is then gradually raised, and the ignition continued with the aid of a Méker or Teclu burner or a blast lamp for at least twenty minutes. The crucible is then cooled in the desiccator and weighed. The ignition over the blast lamp must be continued until the weight is constant.

Much may be said in favour of both forms for weighing calcium. When the quantity of material is fairly considerable it is perhaps best to weigh as carbonate, as the time required to dissociate it to oxide completely is considerable. A small quantity on the other hand is most conveniently weighed as the oxide. It must be borne in mind that calcium oxide is extremely hygroscopic; time must not be wasted when weighing it. The ratios for multiplying the final weight of substance are $\frac{40}{100}$ in the case of the carbonate and $\frac{40}{56}$ in the case of the oxide.

Calcium oxalate is a white powder consisting of microscopic crystals. It is practically insoluble in water and in ammoniacal liquids. It is very sparingly soluble in solutions of weak organic acids, but strong acids such as hydrochloric acid dissolve it easily. It crystallizes from solution with one molecule of water at ordinary temperatures. When heated to 200° C. it becomes anhydrous. As has been previously mentioned, it is converted into the carbonate at higher temperatures.

Determination of manganese

This metal may be determined by several gravimetric processes. In the absence of other metals such as magnesium and zinc, it may be determined with very satisfactory results by precipitation as manganous ammonium phosphate $MnNH_4PO_4$, which is after-

wards converted by ignition into the pyrophosphate $Mn_2P_2O_7$ and weighed as such.

$$2MnNH_4PO_4 = Mn_2P_2O_7 + 2NH_3 + H_2O.$$

In order to determine the percentage of manganese in potassium permanganate, 0·5 to 0·6 gramme of the solid is dissolved in about 100 c.c. of distilled water. A small quantity of a solution of sulphur dioxide is then added to reduce the permanganate to a manganous salt. Excess of a solution of ammonium chloride followed by sodium phosphate and a slight excess of ammonia are then added to the solution of the manganese salt. The manganous ammonium phosphate separates as a gelatinous faintly pink coloured precipitate. The contents of the beaker should be heated for some time in a water bath. Under these conditions the gelatinous precipitate is transformed into a silky crystalline modification, which may be filtered and washed with great ease. The washing may be carried out with hot water as there is practically no danger of hydrolysing the precipitate, but it is safer to use cold water. If the precipitate is crystalline, it may be filtered very easily in a Gooch crucible. The ignition of the precipitate requires a bright red heat. If a filter paper has been used for filtering the precipitate, it is frequently difficult to burn the carbon completely. The addition of a little ammonium nitrate facilitates the complete combustion of the filter paper. The ignition must be continued until the weight is constant.

The weight of precipitate multiplied by $\dfrac{2 \times 55}{2 \times 55 + 2 \times 31 + 7 \times 16}$ will give the weight of manganese in the quantity of salt taken for the analysis. It is necessary to pay careful attention to the conditions of precipitation in preparing manganese ammonium phosphate. The substance should possess a faintly pink colour, not a dark reddish-brown appearance. In the latter case, the precipitate does not correspond to the composition $MnNH_4PO_4$, but is incompletely transformed into the ammonium salt.

Magnesium and zinc may be determined by precipitation as ammonio phosphates followed by ignition to pyrophosphates. The procedure is similar to that above named, but in both cases the precipitate should be washed with dilute ammonia, otherwise low results will be obtained in consequence of hydrolysis. The reciprocal process of determining phosphates can be effected by precipitation with a mixed solution of magnesium sulphate, ammonium chloride and ammonia.

Determination of nickel

At the present time nickel is usually determined by precipitating and weighing the element as the dimethylglyoxime derivative. Dimethylglyoxime,

$$CH_3 . C = NOH$$
$$|$$
$$CH_3 . C = NOH$$
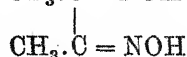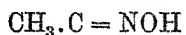
in alcoholic solution is a very sensitive reagent for small quantities of nickel salts in solution, two molecular proportions of the dimethylglyoxime reacting quantitatively with one atomic proportion of nickel, two hydrogen atoms, one from either of the oxime groups in each molecule of the reagent being replaced by an atom of nickel. These two hydrogen atoms form an acid with the acidic radical previously in combination with the nickel salt, and this acid must be neutralized with ammonia to effect complete precipitation.

For practice in the method weigh out about 0·4 gramme of recrystallized nickel ammonium sulphate, $NiSO_4(NH_4)_2SO_46H_2O$, dissolve the salt in about 100 c.c. of distilled water. Then add a slight excess of a 1 per cent. alcoholic solution of dimethylglyoxime (10 c.c. of a 1 per cent. alcoholic solution of the reagent for every 0·1 gramme of the nickel salt may be added) followed by a little ammonia sufficient to be indicated by its odour. Nickel dimethylglyoxime is precipitated quantitatively in accordance with the equation:

$$NiSO_4 + 2C_4H_8N_2O_2 + 2NH_3 = (NH_4)_2SO_4 + C_8H_{14}N_4O_4Ni.$$
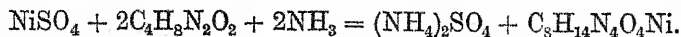
The precipitation should be carried out at the boiling point, and the scarlet precipitate washed by decantation with hot water and filtered through a Gooch crucible. The precipitate is dried at a temperature of about 110° C., cooled in a desiccator and weighed. The weight of precipitate multiplied by $\dfrac{58\cdot7}{288\cdot7}$ will give the weight of nickel in the quantity of substance taken for analysis.

Nickel dimethylglyoxime is a bulky scarlet precipitate, practically insoluble in water and in ammoniacal liquids. It is however soluble in strong acids, and for this reason it is necessary to carry out the precipitation in presence of a slight excess of ammonia. The substance is stable up to temperatures of about 130° C.; at a temperature of about 250° C. it sublimes without decomposition. This method of determining nickel is of the greatest value in such operations as the analysis of nickel steels.

Determination of water of crystallization in hydrated salts

There are two general methods for the determination of water, viz. by loss of weight on heating and by direct weighing of the water after absorption in some desiccating substance. The former method will alone be described in this chapter. In many cases the determination of water is effected indirectly by determining some other constituent of the substance by either a gravimetric or a volumetric method, as, for instance, in the determination of the water of crystallization in hydrated sodium carbonate, p. 74.

When the determination of water is effected by loss of weight on heating, it is essential that the action of heat shall produce no chemical change other than that of the dehydration of the hydrate. The hydrated salt may be weighed out in a crucible or in any other suitable vessel. It should be very finely powdered before weighing. The temperature which is necessary to decompose hydrated salts varies with the nature of the substance; some salt hydrates are readily dissociated at a temperature of 100° C., while others require a higher temperature. In the case of copper sulphate pentahydrate, for example, approximately four of the five molecules of water are removed by keeping the salt at a temperature of 100° C., but in order to remove the last molecule of water the substance must be heated to about 250° C.

There are comparatively few salts which are suitable for practice in the determination of this method of loss of weight; but in addition to copper sulphate, barium chloride which crystallizes with two molecules of water, and magnesium sulphate which contains seven molecules, will be found to give satisfactory results. Barium chloride can be dehydrated completely at a temperature of about 200° C., but magnesium sulphate requires a higher temperature, and should be heated in a crucible over a small flame. Care must be taken not to heat the salt too strongly on account of the danger of conversion into a basic sulphate. About one gramme of the salt should be weighed out for the determination and then heated for about an hour. The substance is then cooled in a desiccator and weighed. The heating and cooling must be repeated until the weight is constant. The result may be expressed as a percentage; but if it is desired to state it in terms of a number of molecules of water, it is necessary to calculate the weight of water which combines with one molecular weight of the anhydrous salt, and then divide this result by the molecular weight of water, viz. 18.

Determination of lead

Lead is most commonly determined by precipitation and weighing as the sulphate. This compound is a heavy white powder, somewhat similar in appearance to barium sulphate, but considerably more soluble in water. At the ordinary temperature one part by weight of lead sulphate will dissolve in about 23,000 parts of water, whereas one part by weight of barium sulphate requires about 400,000 parts of water to dissolve it. Lead sulphate is considerably less soluble in dilute sulphuric acid than in pure water on account of the common ion effect; but if the concentration of acid is much increased, a corresponding increase in solubility of the compound is observed, possibly due to the formation of an acid sulphate. Lead sulphate is considerably less soluble in water containing alcohol than in pure water.

Assuming that there are no substances in solution which are likely to give rise to complications, precipitation should be effected with dilute sulphuric acid of about normal concentration. The precipitate should be washed by decantation with very dilute sulphuric acid, and finally with a *small* quantity of water. Alternatively, after precipitation an equal volume of alcohol may be added to the liquid, and the washing conducted with a mixture of equal parts of alcohol and water, or better with a mixture of alcohol and very dilute sulphuric acid, and finally with a small amount of dilute alcohol. If a filter paper is used, great care must be observed during ignition to avoid reduction of the lead sulphate. Filtration with a Gooch crucible is perhaps more difficult, but is preferable as the risks which attend ignition are more easily avoided. The ignition is concluded at a low red heat, the substance being weighed as $PbSO_4$.

Determination of phosphorus

Phosphorus as orthophosphate is best determined gravimetrically by precipitation as ammonium phosphomolybdate. This compound which has the composition $(NH_4)_3PO_412MoO_36H_2O$ separates as a yellow crystalline precipitate when ammonium molybdate in considerable excess is added to a hot solution of an orthophosphate containing nitric acid and ammonium nitrate. Attention to details of procedure in precipitating the compound is

most important, as otherwise precipitates of variable composition may separate. Certain other anions also form complex acids with molybdic acid, particularly arsenates and vanadates. If these substances are present in solution, special modifications of the procedure are necessary for which larger works should be consulted. The following method recommended by Hillebrand and Lundell* may be adopted when disturbing substances are absent.

The solution should have a total volume of 100 to 200 c.c. and contain about 0·05 gramme of phosphorus present as orthophosphate, 5 to 10 per cent. by volume of nitric acid, and 5 to 15 per cent. of ammonium nitrate. Heat this solution to 40° or 50° C. in a large conical flask and add 15 to 25 times the calculated quantity of ammonium molybdate reagent. This reagent is prepared by mixing 100 grammes of pure molybdic anhydride with 400 c.c. of water, adding 80 c.c. of ammonia solution, and filtering if necessary. Then mix 400 c.c. of nitric acid with 600 c.c. of water. Stir the dilute nitric acid thoroughly with water by a rapid current of air, and add the molybdate solution to this by a tube dipping under the liquid. Continue the stirring with air for one or two hours. The solution should be filtered if necessary. When the molybdate reagent has been added to the phosphate solution, close the flask with a rubber stopper, and shake vigorously for five minutes. Allow the precipitate to settle for at least a quarter of an hour. Then filter through a Gooch crucible and wash with a dilute solution of ammonium nitrate. Finally ignite the precipitate at a red heat. The ultimate product is phosphomolybdic anhydride, $P_2O_5 24MoO_3$, which contains 3·95 per cent. of phosphorus pentoxide.

For practice in the method prepare a solution of microcosmic salt, $NaNH_4HPO_4 4H_2O$, containing from 4 to 4·5 grammes of the salt per litre. To 20 or 25 c.c. of such a solution add 20 or 25 c.c. of normal nitric acid and 20 or 25 c.c. of a 10 per cent. solution of ammonium nitrate. Heat the mixture to about 50° C. in a conical flask. Then add 100 c.c. of a solution of ammonium molybdate prepared by dissolving 50 grammes of ammonium molybdate and 50 grammes of ammonium nitrate in a litre of nitric acid (1 part of nitric acid to 3 parts of water by volume). After shaking

* *Applied Inorganic Analysis*, Chapter XLIV.

thoroughly in the closed flask, filter, wash, and ignite the precipitate as described above.

Although phosphomolybdic anhydride is a stable compound at a red heat, alternative methods of treating the washed precipitate are in common use. An alkalimetric method consists in dissolving the precipitate in excess of standard sodium hydroxide and titrating back with standard acid using phenolphthalein as indicator. If this method is used, precipitation must be carried out at a temperature not above 45° C., and the solution must not be heated *after* the molybdate reagent has been added to the phosphate. The products obtained by dissolving the ammonium phosphomolybdate in sodium hydroxide are normal salts of molybdic acid, R_2MoO_4, and secondary salts of phosphoric acid, R_2HPO_4.

A gravimetric method which is frequently employed consists in dissolving the ammonium phosphomolybdate after washing in dilute ammonia solution and reprecipitating as magnesium ammonium phosphate, $Mg(NH_4)PO_4$, by adding excess of magnesia mixture. Here, too, attention to details is important to secure the production of a precipitate of perfectly definite composition. Neutral magnesia mixture is preferable to the ammoniacal reagent commonly used. The mixture should be prepared by dissolving 50 grammes of magnesium chloride, $MgCl_26H_2O$, and 100 grammes of ammonium chloride in 500 c.c. of water. To this add a *slight* excess of ammonia solution and, after standing overnight, reject any precipitate which may separate. Add dilute hydrochloric acid until the solution is *just* acid and finally dilute to one litre.

The magnesia mixture may be added directly to the warm ammoniacal solution of the phosphomolybdate, but it has been stated* that better results are obtained by acidifying the ammoniacal solution of the phosphomolybdate with dilute hydrochloric acid, adding the magnesia mixture, and finally adding dilute ammonia solution in slight excess. The quantity of magnesia mixture to be added is about 1 c.c. for every centigramme of P_2O_5 in the solution. The liquid must be thoroughly stirred to accelerate the crystallization of the magnesium ammonium phosphate. To ensure complete precipitation of the ammonio-phosphate the liquid

* *Loc. cit.*

should be set aside for some hours before filtering. The washing must be carried out with dilute ammonia solution to suppress any hydrolysis of the precipitate. Finally ignite the precipitate at a bright red heat to magnesium pyrophosphate, $Mg_2P_2O_7$, and weigh as such.

It may be added that although phosphates may be precipitated directly as magnesium ammonium phosphate, the more elaborate procedure which has been described is preferable.

CHAPTER XIV

MODERN DEVELOPMENTS IN VOLUMETRIC ANALYSIS

In the course of the last two decades, considerable advances have been made in developing new methods of analysis of general applicability, in addition to devising new methods which may be useful for some particular object. Perhaps the most fruitful advances have been made in connexion with indicators for oxidation-reduction reactions and for precipitation titrations.

Before discussing some of these newer developments in detail, it may be desirable to say a few words about the unit of volume. The cubic centimetre, for many years accepted as the standard of volume, is a unit which is ultimately based on length, whereas the litre is based on mass. The litre is the volume of one kilogramme of pure water at the temperature of maximum density. It has been shown that the cubic centimetre is very nearly one thousandth part of one litre, but it actually exceeds this value by 28 parts per million. Accordingly the unit of volume which is now adopted is the millilitre, and measuring vessels which are now manufactured have their capacities expressed in terms of this unit.

As the difference between the two units is so small and well within the limits of all ordinary analytical work, it is immaterial which unit is adopted. Nevertheless, it should be pointed out that the National Physical Laboratory has definitely adopted the millilitre as the standard, and has recommended the abandonment of the cubic centimetre.

Preparation of standard hydrochloric acid from the constant boiling mixture

Hulett and Bonner (*J. Amer. Chem. Soc.*, 1909, **31**, 390) have continued the experiments of Roscoe and Dittmar (see page 74), and have found that the method is very satisfactory. Their results were subjected to a very rigid scrutiny by Foulk and Hollingsworth

(*ibid.*, 1923, **45**, 1223) who found that the effect of small variations of pressure has little effect on the composition of the constant boiling mixture. The magnitude of the effect is about one part per thousand for a difference in pressure of 10 mm. The following figures illustrate this.

Pressure (mm. of mercury)	Hydrogen chloride on vacuum weight basis, per cent.
770	20·197
760	20·221
750	20·245
740	20·269

Constant boiling hydrochloric acid is not hygroscopic, and it will maintain its concentration unchanged if it is preserved in a well stoppered bottle and not exposed to direct sunlight. Exposure of acid of this or of greater concentration to direct sunlight will result in slight oxidation of the acid with consequent liberation of chlorine.

Indicators for acidimetric and alkalimetric titrations

Screened methyl orange. Although methyl orange is almost universally used for the titration of strong acids, it cannot be said that the colour change is as readily perceptible, particularly in artificial light, as might be desired. If an inactive blue dyestuff is added to a solution of methyl orange, the colour change from yellow to red is transformed into the approximately complementary colour change from green to violet, which is much more readily perceptible. Various dyestuffs such as indigo or methylene blue may be used for this object, but the best results are obtained by adding 1·4 parts of xylene cyanole FF to one part of methyl orange, the mixture being dissolved in 500 parts of 50 per cent. alcohol. Two or three drops are used for a titration, the liquid being green when alkaline and magenta when acid, these colours being separated by a well defined grey tint corresponding to a *p*H value of 3·8 (Hickman and Linstead, *Journ. Chem. Soc.*, 1922, **121**, 2502).

The sulphone phthalein indicators. Clark and Lubs have made exhaustive studies of the indicator properties of the sulphone phthaleins. These compounds are characterized by striking colour changes, and in most cases over a narrow *p*H range. They have found considerable application for direct determination of the *p*H

value of solutions, but have not come into such general use in analysis.   Mention may, however, be made of bromphenol blue (tetrabromophenolsulphonephthalein) which is blue in alkaline and yellow in acid solution, the pH range being from 3·0 to 4·6.   Another interesting indicator, viz. thymol blue (thymolsulphonephthalein) has a double colour change, namely from red to yellow over the extreme acid pH range from 1·2 to 2·8, and from yellow to blue over the pH range from 8·0 to 9·6.   These indicators may be conveniently used in 0·04 per cent. concentration of their alkali salts. From the values of the pH ranges of these two indicators, it will be evident that bromphenol blue may be used as a substitute for methyl orange, while thymol blue may replace phenolphthalein as far as its alkaline range (8·0 to 9·6) is concerned.   The acid range of thymol blue is practically useless for titration work.   Other indicators of this series which are useful for particular purposes are bromthymol blue (dibromothymolsulphonephthalein) which changes from yellow to blue over a pH range of 6·0 to 7·6, and phenol red (phenolsulphonephthalein) which has a pH range from yellow at a value of 6·8 to red at 8·4.

Nitrazine yellow (2:4 dinitrobenzeneazo-1-naphthol 3:6 disulphonic acid) was described by Wenker (*Ind. Eng. Chem.*, 1934, **26**, 350) as a very useful indicator for work close to the region of neutrality. The colour change is from yellow at pH 6·0 to blue at 7·2, the intermediate range between the values of 6·4 to 6·8 being of a violet grey tint. This indicator is very useful for titration work at low concentrations of strong acids and alkalis.

Note on the action of neutral salts and of alcohol upon indicators

It might be supposed that the colour of an indicator in a solution is determined solely by the hydrogen ion concentration of the solution, and that the presence of other substances, presumably inert, would be without influence upon the colour.   This is not however strictly accurate.   The presence of neutral salts has an appreciable effect upon the colour of indicators, some being more sensitive than others.   The most general effect of neutral salts seems to be to cause displacement of the colour of acid indicators towards the alkaline side and of basic indicators to the acid side. The effect is small, and in all ordinary analytical work where

solutions of deci-normal or higher concentration are used in which the titrations are carried to end-points within a certain definite pH range, it may be wholly neglected.   With more dilute solutions, however, where it becomes necessary to titrate to a particular titration exponent (see page 96), the effect of neutral salts may assume importance.   (See E. Brennecke, "Beseitigung des Titrier-fehlers bei azidi- und alkalimetrischen Titrationen" (*Die chemische Analyse*, Band 33, *Neuere massanalytische Methoden*, pp. 1–19).)

Alcohol lowers the dissociation constant of indicators, but if its concentration in an aqueous solution is small, as is usually the case, its effect may be safely neglected.   The effect of high concentrations of alcohol upon ampholytes, such as amino acids, has been in-vestigated by several investigators, particularly Willstätter and Waldschmidt-Leitz, and it has been shown that the basic function of these classes of compounds is depressed to an extent sufficient to enable them to be titrated as acids with sodium hydroxide, using phenolphthalein as indicator.   Compounds such as glycocoll (aminoacetic acid), may be determined with a fair degree of accuracy by titration with sodium hydroxide, using phenolphthalein as indicator, if a quantity of ethyl alcohol is added to the liquid such that the final volume at the end-point contains about 90 per cent. of that liquid.   Ammonium salts may be determined in the same way.   It is stated that if thymolphthalein is used as the indicator, it is possible to obtain results of a sufficient degree of accuracy for many purposes with a lower concentration of alcohol, possibly as low as 50 per cent.

Titration of strong acids with strong bases at very low concentrations

Reference to the titration curve of hydrochloric acid by sodium hydroxide on page 93 will show that the extent of the horizontal portion of the curve, when the acid and base are of deci-normal concentration, is nearly 8 in pH units, viz. from 3 to 11.   If titration is attempted with the acid and base in centi-normal concentration, the horizontal range will be shortened by at least one pH unit at each end.   This shortening of the range will involve some restriction on the choice of indicators to determine the equivalence point.   It has been estimated that the necessary excess of acid or alkali which must be present in 100 ml. of aqueous solution to give a perceptible

colour change is 0·8 ml. of centi-normal acid or alkali for methyl orange and 0·2 ml. for phenolphthalein, and the writer is of opinion that these figures are an under-estimate.   A further complication arises on account of the difficulty in excluding atmospheric carbon dioxide from the alkali.   In any case it is necessary to titrate to a particular hydrogen ion concentration as discussed briefly on page 96, and nitrazine yellow (pH range 6·0 to 7·2) or a mixed indicator such as bromthymol blue (pH range 6·0 to 7·6) with phenol red (pH range 6·8 to 8·4) will probably be found convenient.

In work of this kind it is absolutely necessary to use a reference solution having a permanent colour to compare with the colour of the indicator which is taken as the end-point.   Nitrazine yellow in changing from the blue (alkaline) to the yellow (acid) colour goes through a violet-grey intermediate tint between the pH range of 6·4 to 6·8.   A dilute solution of chrome alum containing a trace of a cobaltous salt will be found a convenient reference solution for use with this indicator.   The chrome alum must be dissolved in cold water, as heat would cause the compound to be transformed into the green modification, and equilibrium is established very slowly.   The colour of the fresh cold dilute solution is sufficiently similar to that which should be obtained at the end-point of the titration.   A dilute solution of nickel ammonium sulphate containing a little copper sulphate is a useful reference solution for use with the mixed indicator bromthymol blue and phenol red.   The change from violet blue (alkaline colour) to green at pH 7 is easily visible by comparison with this mixture.

It may be added that when $N/100$ silver nitrate is titrated with $N/100$ hydrochloric acid the end-point is easily perceptible if tartrazine is used as an adsorption indicator.   The end-point is clearly visible with a single drop of hydrochloric acid.   Pure silver nitrate (*Analar*) is perhaps the most suitable substance for standardizing hydrochloric acid of very low concentration.

Oxidation and reduction indicators

As a number of reactions which are regularly used in volumetric work are concerned with oxidation and reduction, investigations have been directed to the object of finding substances which undergo striking colour changes at some definite change of potential corre-

sponding to the end-point, as occurs in such a reaction as the oxidation of a ferrous salt in acid solution with potassium dichromate. Of the various substances which may be used for this purpose, two only will be considered in this section, namely barium diphenylamine sulphonate and tri-orthophenanthroline ferrous sulphate. The introduction of these internal indicators is an important improvement on the older method of using potassium ferricyanide as an external indicator, and the compounds may also be used with other volumetric oxidizing agents, such as ceric sulphate.

The mode of action of these modern oxidation-reduction indicators is fundamentally different from that of starch which forms the deep blue adsorption product with iodine and consequently finds successful application in iodometric titration work. It is also different from that of methyl orange which is used in the bromate titration of antimony or arsenic. In these determinations the end-point of the reaction is taken as that point at which the methyl orange becomes oxidized by traces of bromine to colourless products; the process is in short an irreversible one.

It should be noted that reactions concerned with oxidation and reduction are frequently of a more complex character than reactions which involve neutralization. In the latter, the reactions are wholly ionic and the corresponding colour changes of the indicator are brought about by ionic reactions followed by tautomeric changes. The oxidation of a ferrous salt to the ferric condition by ceric sulphate is similar to neutralization inasmuch as the reactions consist simply in the transfer of electrons. Other reactions concerned with oxidation and reduction may involve irreversible chemical changes. A good example of this latter type is the oxidation of an oxalate by potassium permanganate in the presence of dilute sulphuric acid. In that reaction the oxalate anion is completely decomposed to yield two molecules of carbon dioxide.

It has been pointed out that the ideal oxidation-reduction indicator should be an oxidizing or reducing agent which undergoes a completely reversible colour change with a trace of excess of an oxidizing or reducing agent, corresponding to the equivalence point of the reaction, that is to the point at which the quantity of oxidizing agent which has been added to the solution is the quantity which

reacts completely with the oxidizable substance which is being determined. A few words may now be added regarding two indicators which are of practical value.

Barium diphenylaminesulphonate $(C_6H_5NHC_6H_4SO_3)_2Ba$. This compound is best used in a $\frac{1}{3}$ per cent. concentration, about two drops being sufficient for titrating 25 ml. of a reducing agent such as a ferrous salt in $N/10$ concentration with potassium dichromate or ceric sulphate. The change of colour is through greenish grey to violet. This indicator does not give an end-point strictly corresponding to the equivalence point, a small correction being necessary in very exact work. If, however, the oxidizing agent is standardized with this indicator under conditions as similar as possible as those in which it is to be employed, the correction may be neglected.

Tri-orthophenanthroline-ferrous sulphate. This compound which has a deep red colour due to the very stable complex cation $Fe(C_{12}\overset{+}{H_8}\overset{+}{N_2})_3$ was shown by Walden, Hammett and Chapman (*J. Amer. Chem. Soc.*, 1933, **55**, 2649) to approximate more nearly to the requirements of the ideal oxidation-reduction indicator than any other substance employed hitherto. The indicator is prepared by dissolving the calculated quantity of orthophenanthroline hydrate (molecular weight 198) in a 0·025 molar solution of ferrous sulphate containing as little free acid as possible. One drop of such a solution is sufficient for titrating quantities of the order of 25 ml. of a $N/10$ ferrous solution with either ceric sulphate or potassium dichromate. When the reaction is complete the indicator is transformed into the corresponding ferric derivative of orthophenanthroline, which has a pale blue colour, according to the equation:

$$Fe(C_{12}\overset{+}{H_8}\overset{+}{N_2})_3 \rightleftarrows Fe(\overset{+}{C_{12}}\overset{+}{H_8}\overset{+}{N_2})_3 + e.$$
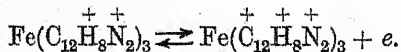
This change takes place at an oxidation potential of 1·14 volts on the hydrogen scale. It has been pointed out that the ideal indicator should change colour at an oxidation potential somewhere between 0·93 volt and 1·26 volts. No correction is necessary for this indicator, and it may be used for a considerable number of titrations which involve reactions of oxidation and reduction.

Adsorption indicators for precipitation titrations

A new type of indicator for determining the end-point of a reaction in which an insoluble precipitate is produced was introduced by Fajans in 1923. Such indicators are particularly valuable for the titration of the halides by silver nitrate and *vice versa*; indeed they have relatively little application apart from determinations of this kind. It is no exaggeration to claim that the introduction of adsorption indicators is the most important advance which has been made in this branch of analysis since the use of potassium chromate by Mohr as an indicator for work in neutral solution, or of Volhard's well-known thiocyanate method for titrating silver in acid solution.

Fluorescein and certain halogenated fluoresceins such as eosin (tetrabromofluorescein) and Rose Bengal (dichlorotetraiodofluorescein) undergo striking colour changes at the end-point when solutions of soluble halides are titrated with silver nitrate, the two first named dyestuffs being suitable for the titration of chlorides and bromides, while Rose Bengal is particularly valuable for the determination of iodides. The colour change with such dyestuffs takes place essentially upon the colloidally dispersed particles or upon the surface of the precipitate, and for this reason they have come to be known generally as adsorption indicators. The use of fluorescein is practically restricted to neutral solutions, but eosin may be used in the presence of weak acids, such as acetic acid, and Rose Bengal can be used in acid solutions which are sufficiently buffered to produce a very low degree of acidity. Other dyestuffs, such as phenosafranine and tartrazine, can be used in the presence of free nitric acid up to a concentration approximating to that of normality.

Experiments have shown that when dilute solutions of a soluble halide and silver nitrate are brought together, the hydrosol of the silver halide acquires a positive charge if the silver ions are present in excess, whereas if the halide ions are present in excess the hydrosol becomes negatively charged. When flocculation takes place, the precipitate adsorbs the corresponding ion. If a dyestuff which is capable of ionizing is present in the solution, either the adsorbed silver ions can combine with the anions of the dye to form a compound, or, alternatively, the adsorbed halide ions may

form a compound with the cations of the dyestuff. The capacity for adsorption of dyestuffs by analogous ions increases with diminishing solubility of the resulting silver compound. Chloride ions have a lower adsorbing power than bromide ions, and these have a lower degree of adsorbing power than iodide ions. The solubilities of the three silver salts in mols per litre are $1\cdot4 \times 10^{-5}$ for the chloride, $0\cdot725 \times 10^{-6}$ for the bromide, and $1\cdot0 \times 10^{-8}$ for the iodide. Dyestuffs also vary as regards capacity for adsorption for a given ion, thus eosin is more strongly adsorbed than fluorescein, and erythrosin (tetraiodo-fluorescein) is so strongly adsorbed as to render it unsuitable for purposes of titration.

Fajans has discussed the colour changes which take place with these indicators in terms of ionic deformation phenomena on the crystal lattice of the silver halides, but alternative explanations based on changes of constitution of the dyestuffs are also possible.

Regarding the practical application of adsorption indicators for titration work, it will be found convenient to use one or two drops of solutions of most of these dyestuffs of $\frac{1}{2}$ per cent. concentration for titrating volumes of 20 to 50 ml. of the halide or of silver nitrate solutions of the order of deci-normal concentration. Phenosafranine should, however, be used in smaller concentration, say $\frac{1}{4}$ per cent. The titrations should be carried out in stoppered bottles with frequent thorough shaking to cause flocculation of the precipitated silver halide. If flocculation does not take place readily, it may be aided by adding a few drops of a solution of a suitable bivalent electrolyte, such as strontium nitrate.

When silver nitrate is run into a dilute solution of a bromide which contains a trace of fluorescein, the lemon colour of the compound persists in the solution until the end-point of the reaction is reached, when the slightest excess of the silver ions causes the precipitate to assume a pale pink colour due to the adsorption of the silver derivative of fluorescein of the surface of the silver bromide. Somewhat similar phenomena are to be observed when eosin is used with either a solution of a chloride or a bromide, the silver eosinate on the precipitate at the end-point having a rose pink colour. When a solution of an iodide is titrated with silver nitrate, using Rose Bengal as indicator, the rose pink colour of the solution persists until the precipitation of silver iodide is complete,

when the further addition of one drop of silver nitrate results in the withdrawal of the dye from the solution, the precipitated silver iodide assuming a fine violet colour. If tartrazine be added to a neutral, or better, to an acid solution of silver nitrate, and a solution of a chloride or a bromide be added from a burette, the silver derivative of this dyestuff which has a buff colour is adsorbed on the surface of the precipitate, but as soon as the reaction has been completed, the slightest excess of halide ions causes the withdrawal of the dye from the precipitate and the supernatant liquid assumes a rich yellowish green colour. Titrations in which tartrazine is used as indicator should always be carried out by running the halide solution into the silver solution, never *vice versa*. Phenosafranine is similar in behaviour to tartrazine as regards applicability in the presence of nitric acid, indeed the presence of free nitrate ions is definitely desirable. Further the colour change which takes place on the surface of the precipitate is equally precise in whichever direction the titration is carried out. When silver ions are in excess the silver halide assumes a fine blue colour, whereas when halide ions are in excess the precipitate is coloured pink. Phenosafranine is, however, more susceptible to interference by other substances in solution than tartrazine. In particular, sulphates must be absent from solutions in which phenosafranine is used as indicator as they prevent the formation of the blue silver derivative of the dyestuff.

It is possible to determine an iodide and a chloride in the same solution by titration with silver nitrate by taking advantage of the much greater insolubility of silver iodide and of the difference in adsorbing capacity of the two anions. Thus if Rose Bengal is added to a neutral solution of the two halides and a solution of silver nitrate delivered from a burette, the end-point when silver iodide is quantitatively precipitated is seen by the withdrawal of the dye from the solution and the production of the violet silver derivative on the surface of the precipitate, the accuracy of the determination being as high as three parts per thousand. The chloride remaining in solution is then determined by filtering off the silver iodide, and continuing the titration of the filtrate and washings, slightly acidified with nitric acid, using phenosafranine as indicator.

It may be stated generally that determinations of silver in acid solution, and consequently of other substances which are determined indirectly in this way, may be effected with as high a degree of exactness as may be realized with Volhard's method. One example may be quoted by way of illustration.

A determination of the percentage of silver in Levol's alloy in approximately $N/100$ concentration as regards the silver content was carried out in the following manner. 0·4424 gramme of pure silver was dissolved in nitric acid and the resulting solution diluted to 500 ml. 0·4736 gramme of the alloy was similarly dissolved in nitric acid and this solution was also diluted to 500 ml. Quantities of 50 ml. were taken for each titration with the following results:

	Volumes of potassium thiocyanate required	Volumes of potassium bromide, using phenosafranine as indicator, required
For the pure silver solution	39·7 ml.	40·95 ml.
For the alloy solution	30·35 ml.	31·2 ml.

The calculated percentages of silver in the alloy are 71·4 by Volhard's method and 71·2 by the adsorption indicator method (see Berry, *Analyst*, 1936, **61**, 315). See K. Fajans, "Adsorptionsindikatoren für Fällungstitrationen" (*Die chemische Analyse*, Band 33, *Neuere massanalytische Methoden*, pp. 161–208).

Ceric sulphate as a volumetric oxidizing agent

In recent years ceric sulphate, $Ce(SO_4)_2$, has been introduced into volumetric analysis as a substitute for potassium permanganate, and it may be stated generally that the compound is capable of effecting all the quantitative oxidations which are commonly carried out in acid solution by the latter reagent. Ceric sulphate has approximately the same oxidizing strength as potassium permanganate in acid solution, and both are stronger than potassium dichromate under these conditions. Acid solutions of ceric sulphate are very stable, and consequently preserve their oxidizing value for considerably longer periods than solutions of potassium permanganate. Further ceric sulphate can be used in the presence of hydrochloric acid, whereas potassium permanganate is useless under such conditions (see page 23). It is however necessary to employ an oxidation-reduction indicator, such as barium diphenyl-

aminesulphonate or tri-orthophenanthroline-ferrous sulphate, in determining the end-point in a ceric sulphate titration in most cases, as the colour change is difficult to determine without such an aid.

A standard solution of ceric sulphate cannot be prepared directly by weighing out the salt, but it is a simple matter to prepare a solution of roughly the desired concentration by dissolving the necessary quantity of the technical product in dilute sulphuric acid, and diluting the resulting solution to one litre. The presence of excess of sulphuric acid is necessary to prevent hydrolysis. The solution has a fine orange yellow colour, similar to that of a solution of potassium dichromate. Ceric sulphate, as usually supplied, is highly impure, and any quantity from 35 to 70 grammes may be necessary to prepare a solution of such a concentration that one litre will be of deci-normal concentration as regards oxidizing power, *i.e.* will contain 0·8 gramme of available oxygen per litre. The solution may be standardized in several ways; for most purposes it will be found convenient to use ferrous ammonium sulphate as has been described for potassium permanganate (see page 17) but with the addition of a few drops of the appropriate indicator.

When a ceric salt is treated with a suitable reducing agent, it is converted quantitatively into a cerous salt, the resulting change of valency being from the quadrivalent to the tervalent condition. If ferrous sulphate is the reducing agent, the reaction may be expressed by the equation:

$$2Ce(SO_4)_2 + 2FeSO_4 = Ce_2(SO_4)_3 + Fe_2(SO_4)_3.$$
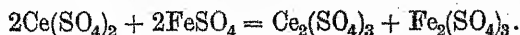
Hydrogen peroxide is oxidized with evolution of oxygen thus:

$$H_2O_2 + 2Ce(SO_4)_2 = Ce_2(SO_4)_3 + H_2SO_4 + O_2.$$
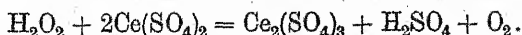
Oxalates are oxidized quantitatively in hot acid solution, *e.g.*

$$H_2C_2O_4 + 2Ce(SO_4)_2 = Ce_2(SO_4)_3 + H_2SO_4 + 2CO_2.$$
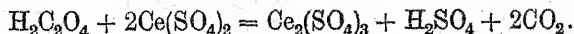
These and other similar reactions may be expressed in general terms as follows:

$$2CeO_2 = Ce_2O_3 + O \text{ (available), or thus } Ce^{IV} = Ce^{III} - e.$$
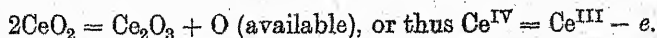
The cerous ion is colourless, and hence results of approximate accuracy may be secured in colourless solutions by adding the

standardized solution of ceric sulphate from the burette until a slight but decided yellow colour is apparent, showing the presence of a trace of the ceric ion. In certain titrations it is definitely desirable to carry out the titration in this way, then to add a drop of a suitable oxidation-reduction indicator, and titrate the small excess of ceric sulphate back with a dilute standard solution of ferrous sulphate.

Direct titration with potassium iodate (Lang's modification)

The method of direct titration with potassium iodate devised by Andrews in which the reaction is caused to take place in presence of a very high concentration of hydrochloric acid (see page 98) is of wide applicability. In this method, the liquid at the end of the titration must consist of about 50 per cent. of concentrated hydrochloric acid by volume in order to suppress any possibility of hydrolysis of the iodine monochloride. In a series of publications from 1922 onwards, Lang has shown that the classes of compounds which may be oxidized quantitatively by Andrews' method may be determined by direct titration with potassium iodate in presence of hydrogen cyanide, and it has been claimed that Lang's method is preferable as a much lower concentration of hydrochloric acid is necessary. The iodine which is liberated in the course of the reaction is ultimately oxidized to iodine cyanide, and the end-point of the reaction is determined with the aid of starch.

The oxidation of potassium iodide by potassium iodate by Andrews' and by Lang's methods is represented by the equations:

$$KIO_3 + 2KI + 6HCl = 3KCl + 3ICl + 3H_2O,$$

and $KIO_3 + 2KI + 3HCl + 3HCN = 3KCl + 3ICN + 3H_2O.$
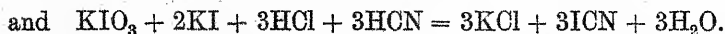
A standard solution of potassium iodate containing about 4 grammes of the salt per litre is a suitable concentration for use with either method. In titrating potassium iodide by Lang's method, about 50 ml. of $2N$ hydrochloric acid and 5 ml. of a 10 per cent. solution of potassium cyanide are added, together with a small quantity of starch solution. The solution of potassium iodate is then added from the burette until the blue colour vanishes. The titrations should be carried out in long necked flasks to avoid inhalation of hydrogen cyanide vapour as far as possible.

The writer is definitely of opinion that the advantages which have been claimed for Lang's method over that of Andrews are exaggerated. There are very few substances which cannot be determined with equal facility by both methods. Moreover the end-point in a Lang titration is sometimes rather difficult to determine with exactness. In particular cases, where there may be special advantages to be secured by avoiding the use of a very high concentration of hydrochloric acid, Lang's method may be desirable. Arsenic and antimony are both oxidized from the tervalent to the quinquevalent condition by potassium iodate in hydrochloric acid solution, and both methods of procedure may be followed; but the acid concentration should certainly be lower than is usual in an Andrews titration, about half to two-thirds of the normal quantity of hydrochloric acid being suitable. The oxidation of arsenious chloride takes place according to the equation:

$$KIO_3 + 2AsCl_3 + 5H_2O = 2H_3AsO_4 + KCl + ICl + 4HCl.$$
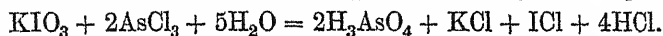
If this titration is attempted with too high a concentration of hydrochloric acid, the reaction is very slow. On the other hand, too low a concentration is undesirable owing to the risk of hydrolysis of the iodine monochloride. On this account, Lang's method of procedure may be preferable.

It is sometimes desirable to start an iodate titration by adding a small quantity of iodine monochloride solution (about $0.005 M$) to the reducing agent. This is prepared by dissolving 0.3067 gramme of potassium iodide and 0.1975 gramme of potassium iodate in a mixture of 250 ml. of pure concentrated hydrochloric acid and 250 ml. of water. The solution must be preserved in a dark glass bottle.

Reduction with liquid amalgams

A useful and accurate method for effecting the quantitative reduction of a number of substances from a higher to a lower state of oxidation was introduced by Nakazono in 1921. The method consists in shaking the solution of the substance, acidified with dilute sulphuric acid with a liquid amalgam for a short time (usually about one minute). A zinc amalgam containing about 2 per cent. of zinc will be found convenient for most purposes. With this reagent, ferric salts are reduced to the ferrous condition,

vanadic acid or a vanadyl salt is reduced to a vanadous salt, uranyl salts are reduced to the uranous condition, and similar results are obtained with titanic and ceric salts. Iodates and bromates are reduced completely to iodides and bromides respectively. It is even possible to make use of amalgams which effect reductions to different stages. Thus according to Someya, vanadic acid is reduced to vanadous sulphate when zinc amalgam is used, whereas bismuth amalgam effects reduction to the vanadyl stage but no further.

The reductions are most conveniently carried out in well stoppered bottles, about 30 to 50 ml. of amalgam being a convenient quantity for reducing volumes of 50 ml. at a time of solutions of the order of deci-normal concentration. The same amalgam can be used a great number of times before it becomes exhausted. After reduction is complete, the contents of the bottle are transferred to the titration flask or other vessel, and the bottle rinsed out two or three times with small quantities of water, the rinsings being of course added to the flask. The method is greatly superior to the old fashioned method of using granulated zinc; indeed it would now be correct to describe the use of granulated zinc as obsolete.

It will be seen in what follows how some of these newer methods are applied to various analytical problems. Some examples of other methods which have been long in use, and of improvements in the older processes are also included.

The reaction between arsenious oxide and iodine

Washburn (*J. Amer. Chem. Soc.*, 1908, **30**, 31) has made an exhaustive study of the reaction between an alkaline arsenite and iodine, and has found that when certain conditions are observed, results of extreme accuracy can be obtained. The most important condition is the maintenance of the hydrogen ion concentration of the solution between the pH values of 4 and 9. If sodium bicarbonate is used, as described on page 41, the liquid should be kept saturated with carbon dioxide. A better method of preparing a standard solution of sodium arsenite is to make up the solution with a mixture of disodium hydrogen phosphate and sodium dihydrogen phosphate in the proportion of two mols of the former to one of the latter instead of using sodium carbonate. In order

to prepare a deci-normal solution of sodium arsenite, dissolve 4·945 grammes of pure arsenious oxide of *Analar* quality in a solution of 12 grammes of pure sodium hydroxide in 200 ml. of water. To this solution add 10 ml. of syrupy orthophosphoric acid in 100 ml. of water, and finally dilute the whole solution to one litre. Such a solution will preserve its titration value indefinitely, and is highly reliable for standardizing solutions of iodine. Its pH value is within the necessary limits, as may be shown qualitatively with indicators, namely alkaline to methyl orange and acid to phenolphthalein.

In all iodometric work in which starch is used as the indicator, the starch solution should be freshly prepared. Many chemists prefer to use Lintner's soluble starch as it gives a clear solution in boiling water. The end-point colour when a trace of iodine is present in excess is of a violet tint, somewhat different from the pure blue obtained with ordinary starch.

Standardization of sodium thiosulphate solutions with potassium ferricyanide

On account of its high equivalent weight (329·1), ready solubility in water, and the ease with which it can be obtained in highly pure condition, this salt is particularly suitable for standardizing solutions of sodium thiosulphate.

The reaction $K_3Fe(CN)_6 + KI = K_4Fe(CN)_6 + I$ is reversible. In dilute acid solution it proceeds from left to right, more rapidly, however, in more concentrated acid solution. It is necessary to remove the ferrocyanide produced in the reaction by precipitation as insoluble zinc ferrocyanide to enable the substance to be employed satisfactorily for the present purpose. Kolthoff recommends the following procedure.

The substance is dried at 100° C., and a standard solution prepared by dissolving the necessary quantity of the salt in water, and diluting the solution to the required volume. For each gramme of potassium ferricyanide, 30 ml. of water, 10 ml. of N potassium iodide solution, and 8 to 10 ml. of N hydrochloric acid are added. Then 10 ml. of a 30 per cent. solution of zinc sulphate are added to the mixture. Zinc ferrocyanide separates as a gelatinous precipitate. The solution of sodium thiosulphate is

11—2

added until the colour of the iodine is nearly discharged, and starch solution is added towards the end of the titration. The titration is then continued until the blue colour vanishes.

Determination of arsenic as silver arsenate

Arsenates in solution are precipitated quantitatively as silver arsenate, Ag_3AsO_4, provided the solution is either neutral, or if acid, the free hydrogen ions must be kept at a small concentration by having the solution well buffered with sodium acetate. Arsenites must first be oxidized by treatment with nitric acid. If much free nitric acid is present, the excess of free acid must be neutralized with sodium hydroxide. The solution must not be alkaline, because the addition of silver nitrate would result in the precipitation of silver oxide together with silver arsenate. Good results are obtained by just acidifying an alkaline solution with dilute nitric acid and adding a moderate quantity of a saturated solution of pure sodium acetate. Excess of a solution of silver nitrate is then added, and the silver arsenate washed free from excess of silver nitrate.

The washed silver arsenate is dissolved from the filter with dilute nitric acid of about normal concentration, and the silver in the acid solution is then determined either by Volhard's method or by titration with a standard solution of potassium bromide using phenosafranine or tartrazine as an adsorption indicator. It will be evident that three atomic proportions of silver are equivalent to one of arsenic.

It may be added that this method should not be employed for the determination of arsenic in mixtures without ascertaining the absence of other substances which are precipitated under similar conditions with silver nitrate. In particular, phosphates and chromates are precipitated under these conditions.

Determination of chromium by oxidation to dichromate with ammonium persulphate

Chromic salts may be oxidized to dichromates by boiling with a solution of ammonium persulphate. The oxidizing agent must be present in excess, and silver nitrate must be present to catalyse the reaction. The excess of persulphate is destroyed by prolonging the boiling for a short time after oxidation of the chromium to the

dichromate condition has been judged to be complete. After cooling, the dichromate solution is diluted to a definite volume, and its concentration determined with reference to a standard solution of ferrous sulphate. The procedure may be illustrated with reference to an experiment on the determination of chromium in chrome alum, $K_2SO_4Cr_2(SO_4)_3 24H_2O$.

2·5 grammes of chrome alum were dissolved in 50 ml. of water. Then 20 ml. of a $N/10$ solution of silver nitrate followed by 50 ml. of a 10 per cent. solution of potassium persulphate were added. The liquid was boiled gently for a quarter of an hour. After thorough cooling, the solution was diluted to 200 ml.

A solution of ferrous ammonium sulphate, corresponding to an iron content of 2·875 grammes of the metal per litre, was prepared, and aliquot parts of the iron solution titrated with the dichromate solution, using potassium ferricyanide as an external indicator.

20 ml. of the ferrous solution required 13·8 ml. of the dichromate solution for complete oxidation. From this result, it follows that the weight of iron which was oxidized by the chromium (as dichromate) in 2·5 grammes of chrome alum $= \dfrac{4 \times 2·875}{13·8}$ or 0·833 gramme. This corresponds to a weight of 0·258 gramme of chromium. The percentage of chromium in the compound is therefore 10·3, the value calculated from the formula being 10·4 per cent.

Determination of antimony with potassium bromate

At a temperature of about 80° C., and in the presence of dilute hydrochloric acid antimony is oxidized quantitatively from the tervalent antimonious to the quinquevalent antimonic condition by potassium bromate according to the equation:

$$KBrO_3 + 6HCl + 3SbCl_3 = KBr + 3H_2O + 3SbCl_5.$$
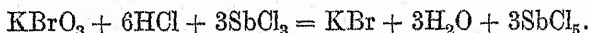
From this equation it is evident that the equivalent weight of potassium bromate is one-sixth of its molecular weight. A deci-normal solution of the compound will therefore contain 7·283 grammes of the compound per litre. Potassium bromate of *Analar* quality may be used to prepare the standard solution directly, but if the salt is not of this degree of purity the solution should be standardized on a solution containing a known quantity of antimony under conditions as identical as possible as those in which it is to be employed.

The end-point of the reaction is determined by adding a few drops of a solution of methyl orange to the acidified antimony solution. As long as an insufficient quantity of potassium bromate has been added from the burette to complete the oxidation of the antimonious salt, the red colour of the methyl orange will persist in the solution, but as soon as the reaction is completed, the slightest excess of potassium bromate will liberate a trace of bromine from the potassium bromide formed in the reaction, with the result that the methyl orange is immediately oxidized to colourless products. The liquid should be well swirled during the titration to avoid local excesses of bromate, and the titration should be carried out slowly particularly towards the end of the reaction.

If the antimony is present originally in the higher state of oxidation, it must first be reduced to the antimonious condition by boiling with sulphur dioxide. This may be effected by adding one or two crystals of sodium sulphite to the solution of the antimony compound acidified with dilute hydrochloric acid, the boiling being continued until the whole of the sulphur dioxide has been expelled from the solution. Arsenic may be determined in the same way as antimony, but it should be remarked that solutions which contain tervalent arsenic acidified with hydrochloric acid are liable to lose arsenic when boiled on account of the volatility of arsenious chloride. If it is necessary to reduce arsenates with sulphur dioxide, the boiling must not be carried out in an open flask, but a reflux condenser must be used. There is practically no risk of loss of arsenious chloride at the temperature necessary for titration with potassium bromate.

The analysis of ammonium carbonate

Besides normal ammonium carbonate, $(NH_4)_2CO_3$, and ammonium bicarbonate, NH_4HCO_3, other products are known having variable ratios of ammonia to carbon dioxide. Technical ammonium carbonate is chiefly a mixture of the bicarbonate and ammonium carbamate, $NH_4CO_2NH_2$. The analysis of these various compounds involves the determination of the ammonia and the carbon dioxide.

A standard solution of the sample is prepared. It will usually be convenient to work with a solution of normal or semi-normal

concentration.   The ammonia is determined first by direct titration with a standard acid solution using screened methyl orange as indicator.   Another measured quantity of the original solution is boiled with a known quantity (excess) of a standard solution of sodium hydroxide, which must be free from carbonate, until all ammonia has been expelled from the solution.   The solution will now contain a mixture of sodium hydroxide and sodium carbonate, the sodium carbonate being equivalent to the carbon dioxide previously combined with the ammonia.   The sodium hydroxide and sodium carbonate are then determined by titration with a standard acid solution, using phenolphthalein to obtain the end-point corresponding to the sodium hydroxide and one-half of the sodium carbonate, and screened methyl orange to obtain the total alkali as described on page 75.

Determination of hydrochloric acid and ammonium chloride in a solution

The hydrochloric acid was first determined by titration with standard sodium hydroxide using screened methyl orange as indicator.

25 ml. of the solution required 8·9 ml. of sodium hydroxide, from which it follows that the solution contained 6·94 grammes of hydrochloric acid per litre.

To 25 ml. of the solution 8·9 ml. of sodium hydroxide solution were added to neutralize the hydrochloric acid.   Then 4 ml. of 20 per cent. formaldehyde solution were added, and the liquid diluted with about an equal volume of water.   The mixture was heated to 50° C. for two minutes to convert the ammonium chloride into hexamethylene tetramine and hydrochloric acid, and titrated with sodium hydroxide using phenolphthalein as indicator.

10·1 ml. of sodium hydroxide were required, from which it follows that the solution contained 11·54 grammes of ammonium chloride per litre.

In another experiment the procedure was varied by adding a measured excess of sodium hydroxide solution after heating with formaldehyde to 50° C. for two minutes, and determining the excess of sodium hydroxide which remained after the reaction by titration with standard hydrochloric acid.   The same titration value for the

sodium hydroxide equivalent to the ammonium chloride was found, viz. 10·1 ml.

The mixture was diluted to one-quarter of its original concentration, and the total chloride present determined by titration with a standard solution of silver nitrate (14·04 grammes per litre) using phenosafranine as indicator. 20 ml. of the dilute mixture required 24·6 ml. of silver nitrate, from which the total chloride concentration is 14·4 grammes per litre.

The total chloride concentration as calculated from the sodium hydroxide titrations is also 14·4 grammes per litre.

Determination of potassium iodate and potassium iodide in a mixture

A solution containing 2 grammes of the iodate and 9 grammes of the iodide per litre was prepared. The iodate was determined by causing 25 ml. of the mixture to liberate iodine from excess of acidified potassium iodide, and titrating the iodine with a standard solution of sodium thiosulphate. The volume of thiosulphate required was 14·1 ml. of a solution containing 15·7 grammes of $Na_2S_2O_3$ per litre. The calculated value of the weight of potassium iodate from this experiment is 2·0 grammes per litre.

The iodide was then determined by direct titration of 25 ml. of the mixture with a standard solution of potassium iodate in presence of a high concentration of hydrochloric acid, using chloroform to determine the end-point of the reaction when the iodine was completely oxidized to iodine monochloride as described on page 98. 24·5 ml. of a solution of potassium iodate containing 3·82 grammes of the salt per litre were required.

In calculating the weight of potassium iodide per litre, it must be remembered that account must be taken of the iodate in the mixture. The weight of this salt in 25 ml. of the mixture is 0·05 gramme. The weight of potassium iodate delivered from the burette is 24·5 × 0·00382 or 0·0936 gramme. It follows therefore that 0·1436 gramme of potassium iodate has reacted quantitatively with the potassium iodide in 25 ml. of the mixture, and since the reaction takes place according to the equation

$$KIO_3 + 2KI + 6HCl = 3KCl + 3ICl + 3H_2O,$$
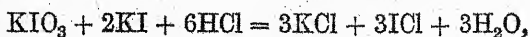
214 parts by weight of potassium iodate react with 2×166 parts by weight of potassium iodide. The calculated weight of potassium iodide from this experiment is 8·90 grammes per litre.

Determination of sodium sulphite and sodium thiosulphate in a mixture

Sulphites and thiosulphates are both oxidized quantitatively by iodine, the former being converted into sulphates, the latter into tetrathionates. If however excess of formaldehyde is added to a dilute aqueous solution of the two salts, the bisulphite derivative of formaldehyde is produced together with an equivalent quantity of sodium hydroxide, according to the equation

$$Na_2SO_3 + CH_2O + H_2O = CH_2(OH)SO_3Na + NaOH.$$
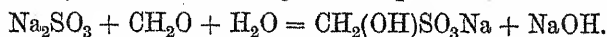
If excess of dilute acetic acid is added to the liquid, the resulting solution contains the unchanged thiosulphate, since this substance does not react with formaldehyde. Two titrations with respect to iodine are therefore required to determine the two constituents.

In the following example, standard solutions containing 11·8 grammes of iodine per litre and 16·6 grammes of sodium thiosulphate per litre were used.

50 ml. of the iodine solution required 44·3 ml. of sodium thiosulphate.

50 ml. of the iodine solution together with 25 ml. of the mixture required 20·3 ml. of sodium thiosulphate.

25 ml. of the mixture were treated with 10 ml. of a 10 per cent. solution of formalin, and then acidified with dilute acetic acid. 50 ml. of iodine solution were added, and this required 29·2 ml. of thiosulphate.

The volume of standard thiosulphate which is equivalent to the sodium thiosulphate in 25 ml. of the mixture is clearly 15·1 ml., and this is equivalent to 17·05 ml. of iodine. The calculated concentration of sodium thiosulphate in the mixture from this experiment is 10·0 grammes per litre.

The volume of standard thiosulphate which is equivalent to the sodium sulphite in 25 ml. of the mixture is clearly 8·9 ml., which is equivalent to 10·05 ml. of iodine. The calculated concentration of sodium sulphite (anhydrous) in the mixture from this experiment is 2·35 grammes per litre.

The mixture for this experiment was prepared with the addition of 2 per cent. of ethyl alcohol (20 ml. per litre) in order to retard the oxidation of the sodium sulphite by atmospheric oxygen. If the alcohol is omitted, oxidation takes place somewhat rapidly.

Determination of permonosulphuric acid (Caro's acid) in the presence of perdisulphuric acid

Persulphates (salts of perdisulphuric acid, $H_2S_2O_8$) may be readily determined by allowing a measured quantity of the solution of the salt to react with a measured excess of ferrous sulphate solution, having a known titration value towards a standard solution of potassium permanganate for a short time, and subsequently determining the amount of ferrous sulphate which remains unoxidized by a second titration with potassium permanganate. The oxidation of the ferrous sulphate takes place according to the equation

$$K_2S_2O_8 + 2FeSO_4 = K_2SO_4 + Fe_2(SO_4)_3.$$

When a persulphate is treated with concentrated sulphuric acid at $0°$ C. and the liquid poured on to crushed ice, the resulting solution is found to have much more powerful oxidizing properties than the original persulphate. This is due to the formation of Caro's acid, H_2SO_5, which is produced by the hydrolysis of the perdisulphuric acid,

$$H_2S_2O_8 + H_2O = H_2SO_5 + H_2SO_4.$$
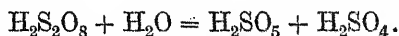
Caro's acid will liberate iodine *immediately* from a solution of potassium iodide, whereas perdisulphuric acid will only do so very slowly. Caro's acid will also liberate bromine quantitatively from a solution of potassium bromide, and will also oxidize a solution of a sulphite quantitatively to sulphate. These two latter reactions are of value for determining the concentration of Caro's acid in a solution which contains both persulphuric acids. The total available oxygen in the solution is best determined by the ferrous sulphate and permanganate method already described.

It is instructive to compare the results obtained by the potassium bromide and sodium sulphite methods for reducing a solution of Caro's acid. This may be done as follows. Approximately 6 grammes of potassium persulphate are triturated with concentrated sulphuric

acid (about 10 or 12 ml.) for about half an hour at 0° C.   The liquid is then poured on to crushed ice, and the resulting solution (which will of course contain some unchanged persulphate) diluted to a known volume (200 ml.).   If the solution has been prepared carefully, it should be practically free from hydrogen peroxide, but if the temperature has been allowed to rise, it will give a very decided reaction with titanic sulphate.   Hydrogen peroxide is produced by hydrolysis of some of the permonosulphuric acid thus:

$$H_2SO_5 + H_2O = H_2SO_4 + H_2O_2.$$
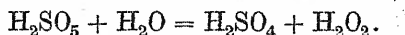
To determine the Caro's acid by the bromide method, excess of potassium bromide is added to a measured quantity of the solution, and the bromine which is liberated is determined by titration with a standard solution of sodium arsenite.   The arsenite is oxidized quantitatively to arsenate, and the end-point of the reaction is determined by adding a drop of a solution of methyl orange to the solution from time to time.   Free bromine immediately oxidizes azo dyestuffs, such as methyl orange, to colourless products.   The end-point of the reaction is therefore that point at which the red colour persists,

$$As_2O_3 + 2Br_2 + 2H_2O = As_2O_5 + 4HBr.$$

The sulphite method is carried out as follows.   A solution of sodium sulphite of approximately $N/10$ concentration is prepared, and stabilized by adding 2 per cent. of its volume of ethyl alcohol. The presence of the alcohol renders the solution considerably less sensitive to oxidation by the air.   The solution is standardized by running it from a burette into a measured quantity of a standard solution of iodine, no indicator being present, two atomic proportions of iodine being equivalent to one molecular proportion of sodium sulphite.   Two drops of a solution of iodine are then added to a measured quantity of the solution of Caro's acid, and the standardized solution of sodium sulphite delivered from the burette until the colour of the iodine is just discharged.   The sulphite is oxidized according to the equation

$$H_2SO_5 + Na_2SO_3 = 2NaHSO_4.$$

A comparison of the results obtained by determining Caro's acid in a solution prepared as described above using (a) sodium

sulphite and (b) potassium bromide as the reducing agent gave the following results.

20 ml. of a solution of iodine required 16·4 ml. of a solution of sodium sulphite. The iodine solution contained 12·12 grammes of the element per litre, and the sodium sulphite solution contained 2 per cent. of ethyl alcohol. From this it follows that the concentration of the sodium sulphite solution was 7·33 grammes per litre.

20 ml. of the solution of Caro's acid with two drops of iodine required 15·6 ml. of sodium sulphite. From this it follows that the concentration of Caro's acid was 5·15 grammes per litre.

To 20 ml. of the solution of Caro's acid, excess of potassium bromide was added. The liberated bromine was determined by titration with standard sodium arsenite solution (5·45 grammes of As_2O_3 per litre), 16·0 ml. were required, using methyl orange as indicator. From this experiment the concentration of Caro's acid was 5·02 grammes per litre.

If it is required to determine hydrogen peroxide in presence of either or both of the persulphuric acids, it has been shown that this cannot be done accurately by titration of the peroxide with potassium permanganate. It is generally agreed that hydrogen peroxide and Caro's acid do not react together to an appreciable extent in dilute solution, but when potassium permanganate is added, it induces a reaction involving oxidation of some of the hydrogen peroxide at the expense of the Caro's acid. Accordingly the determination of the hydrogen peroxide, as calculated from the permanganate titration, gives rise to low results. The writer has, however, found that accurate results can be obtained by substituting ceric sulphate for potassium permanganate. At the ordinary temperature, titrations of hydrogen peroxide with ceric sulphate in the presence of Caro's acid give rise to irregularities similar to though appreciably smaller than those encountered with potassium permanganate. When, however, the titrations are carried out at 0° C. by placing a few fragments of ice in the titration flasks, accurate results are obtained. The best results are obtained by placing a little ice in the flask, adding about 1 ml. less than the volume of ceric sulphate solution necessary to oxidize the hydrogen peroxide in the mixture (as found by a previous direct experiment), then adding the measured volume of liquid taken for analysis, and

finally completing the titration, no indicator being present. The two persulphuric acids are then determined separately as described above. Caro's acid may also be determined by allowing the solution to react with a measured excess of a solution of vanadyl sulphate, which it oxidizes quantitatively to vanadic acid at the ordinary temperature, perdisulphuric acid being without action upon vanadyl sulphate under these conditions. The excess of vanadyl sulphate is then determined by a standard solution of potassium permanganate (Berry, *Analyst*, 1933, **58**, 464).

CHAPTER XV

THE ANALYSIS OF GASES

The quantitative analysis of gases is for the most part carried out by volumetric methods. The term *volumetric* in this connexion refers to the measurement of gas volumes. Other methods, such as gravimetric methods for the determination of carbon dioxide and colorimetric methods for certain gases such as carbon monoxide, are also employed. The fundamental experiments for analysing gases by volume measurements were made by Cavendish. Later Bunsen developed gasometric methods to a high degree of refinement, and in more modern times the subject has been greatly extended, more particularly with regard to improvement and elaboration of apparatus.

The two main aspects of quantitative analytical work on gases namely the determination of the volume of a gas produced in a chemical reaction and the analysis of gaseous mixtures by treatment with reagents will be considered separately. The former type of determination requires no special apparatus apart from a suitable gas burette to enable the volume of a gas produced in the experiment to be measured with sufficient accuracy. For the quantitative analysis of gaseous mixtures, and this is by far the larger part of gas analysis, many forms of apparatus have been devised. Indeed it would be correct to say that in this branch of analytical chemistry more advances have been made from refinement of apparatus than from the discovery of new reactions.

Determination of the volume of a gas evolved in a reaction

Of the various forms of apparatus which have been devised for gas volumetric work, one of the simplest and most satisfactory is the Ostwald gas burette (Fig. 7). The use of this apparatus may

perhaps be most simply illustrated with reference to the deter-
mination of hydrogen peroxide in aqueous solution by catalytic
decomposition with colloidal platinum and measurement of the
volume of oxygen thus obtained. The concen-
tration of hydrogen peroxide is usually stated
practically in this way. Thus five volume
hydrogen peroxide denotes a solution of such
concentration that a given volume of the liquid
will when completely decomposed produce five
times its own volume of oxygen. To carry out
a determination, a given volume of the solution,
20 ml. of peroxide of five-volume concentration
is a convenient quantity, is placed in the flask.
The small test tube contains 2 or 3 ml. of
colloidal platinum. The joints are then made
air-tight and the level of the water in the tubes
A and B is adjusted. It is best to start with the
water level a few ml. from the top of the
tubes rather than to attempt to bring the
reading to the zero point. The level in the

Fig. 7.

tube A is read when the whole apparatus is at the temperature of
the room which is shown by the water levels remaining constant.
The temperature and pressure are carefully noted. The reaction
is started by tilting the flask which results in mixing the colloidal
platinum with the hydrogen peroxide. Oxygen is rapidly evolved,
and when the reaction is completed the water levels are again
adjusted. If it has been necessary to heat the flask, the apparatus
must be allowed to regain the temperature of the room as indicated
by constancy of the water levels. The difference between the new
volume and the original volume is clearly equal to the volume of
oxygen which has been evolved. The temperature and pressure
are noted, and, taking account of the pressure of water vapour,
the volume of the gas is reduced to normal temperature and
pressure. The result may also be expressed in terms of weight by
using the gramme molecular volume (22·4 litres).

It may be stated in general terms that the Ostwald gas burette
may be used for the measurement of any sparingly soluble gas
which is evolved in a reaction. Thus urea in aqueous solution may
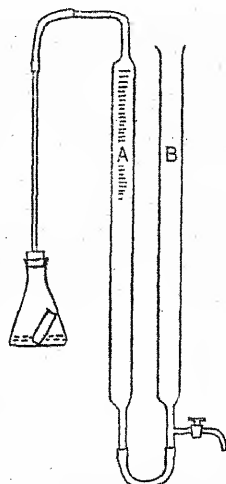
be determined by decomposition with sodium hypobromite according to the equation

$$CON_2H_4 + 3NaOBr = 3NaBr + 2H_2O + CO_2 + N_2.$$
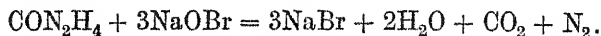
The carbon dioxide dissolves in the alkali present in the solution of sodium hypobromite, and pure nitrogen is collected in the gas burette. The volume of nitrogen obtained in this reaction is however not quantitative, a constant deficit of about 8 per cent. being invariably observed. Much discussion has taken place regarding the cause of this deficiency, and it would appear probable that the explanation is to be traced to the ammonium cyanate which is in equilibrium with the urea reacting differently with the hypobromite, nitrogen not being evolved from cyanates.

Two other forms of apparatus which are in general use may be mentioned here. Schiff's nitrometer is a gas burette consisting of a graduated glass tube provided with a tap at the top and a mercury trap at the bottom. The apparatus is used in connexion with Dumas' method for the determination of nitrogen in organic compounds. The substance is oxidized by heating with copper oxide, and the mixture of carbon dioxide and nitrogen passed into the nitrometer, which is charged with a concentrated solution of potassium hydroxide. The carbon dioxide is absorbed by the potash, and the nitrogen collected in the nitrometer. As the gas is collected over concentrated potash, the correction for the pressure of water vapour may be neglected as the vapour pressure of concentrated solutions of potash is considerably lower than that of pure water at the same temperature. Details for carrying out a determination of nitrogen by Dumas' method may be found in any text-book of practical organic chemistry, and it is therefore unnecessary to discuss them here, but it may be added that the Schiff nitrometer could be used in other types of work in which the quantitative separation of a sparingly soluble gas from one which is readily absorbed by a liquid reagent is required.

Lunge's nitrometer, which is much used for the analysis of nitrates and was originally devised in connexion with the analysis of nitrous vitriol, consists of an apparatus in which the nitrate is allowed to react with mercury and concentrated sulphuric acid within the measuring vessel. The nitrate under these conditions

is quantitatively reduced to nitric oxide according to the equation $6Hg + 3H_2SO_4 + 2HNO_3 = 3Hg_2SO_4 + 4H_2O + 2NO$.

The volume of nitric oxide which is evolved is therefore a direct measure of the nitrate.   Details for the use of this apparatus must be sought in larger works.

Analysis of gaseous mixtures by successive absorption with reagents

Many forms of apparatus have been devised for the quantitative analysis of gaseous mixtures by successive absorption with reagents since the pioneer work of Bunsen in the nineteenth century. Although a high degree of accuracy was realized by Bunsen's methods, they are never employed at the present time as the processes are much too slow.   Of the modern methods those devised by Hempel are the most important, as the analysis can be effected rapidly and with sufficient accuracy for technical purposes.   Before describing Hempel's apparatus in detail, we must discuss the reagents which are usually employed as absorbents for the commoner gases.

Carbon dioxide. This gas is always determined by absorption with a concentrated solution of potassium hydroxide.   A convenient concentration is 33 per cent.   Sodium hydroxide may also be used, but is less satisfactory as the sodium carbonate formed in the reaction is less soluble than potassium carbonate.

Oxygen. A number of reagents has been used for the absorption of this gas.   An alkaline solution of pyrogallol is perhaps most commonly employed.   The gas dissolves fairly rapidly in this reagent with formation of dark brown oxidation products. Alkaline pyrogallol is, however, by no means an ideal reagent, as the solution is stated to give off traces of carbon monoxide under certain conditions.   An alkaline solution of sodium hydrosulphite, prepared by dissolving 50 grammes of the salt in 250 ml. of water and adding 40 ml. of a concentrated solution of sodium hydroxide (500 grammes of sodium hydroxide in 700 grammes of water) is a satisfactory absorbent, much cleaner than alkaline pyrogallol, but its action is definitely slower.   Phosphorus in the form of sticks partly immersed in water is a satisfactory absorbent.   As the oxidation

products of phosphorus are completely soluble in water and the absorption under ordinary conditions is rapid, results of a high degree of accuracy are attainable. There are, however, certain points to be noted in connexion with the use of phosphorus. In the first place, the apparatus containing the element must be shielded from light as far as possible, as otherwise a film of red phosphorus gradually develops on the sticks and its activity is greatly diminished in consequence. Secondly, the presence of certain organic compounds such as ethylene, even in very small concentration, inhibit the reaction between oxygen and phosphorus. Thirdly, if the partial pressure of oxygen in a gaseous mixture exceeds a certain critical concentration, oxidation is stopped. If this contingency arises, it is necessary to dilute the mixture with a known quantity of nitrogen.

Carbon monoxide. This gas is usually absorbed by cuprous chloride either in hydrochloric acid or in ammoniacal solution, the latter being preferable. Several different formulae have been suggested for preparing suitable solutions of ammoniacal cuprous chloride of which the following may be quoted as an example. Dissolve 40 grammes of cuprous chloride in 125 ml. of concentrated hydrochloric acid. To this solution add gradually 130 ml. of concentrated ammonia solution, cooling the flask during the addition of the ammonia. Then add a small further quantity of ammonia until the white precipitate just dissolves; a large excess of ammonia should be avoided. The absorption of carbon monoxide by solutions of cuprous chloride is due to the formation of addition compounds of the type $CuCl, CO, 2H_2O$.

On account of the highly poisonous character of carbon monoxide, much attention has been given to the development of methods for detecting and determining small quantities of this gas. A colorimetric method depending on the reduction of a solution of palladium chloride,

$$PdCl_2 + CO + H_2O = Pd + 2HCl + CO_2,$$
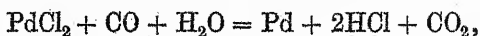
may be quoted as an example.

A better method for determining small quantities of carbon monoxide depends upon the reaction between this gas and haemoglobin. A very stable compound, carboxyhaemoglobin, is formed

by the union of carbon monoxide and the colouring matter of the blood. The colour of normal blood when diluted with water is yellow, whereas that of blood which has absorbed carbon monoxide is pink. The absorption spectra of oxyhaemoglobin and of carboxyhaemoglobin are similar, both having two bands in the yellow and green but there is a slight difference in their position. Spectroscopic methods may be used for determining the mean wave-lengths of the two bands and therefore of measuring the relative proportions of the two compounds. It has been stated that colorimetric methods are more sensitive for quantitative work.

Another reaction which has been much used for the determination of carbon monoxide is the reaction with iodine pentoxide. At temperatures between 120° and 200° C. the following reaction takes place,

$$5CO + I_2O_5 = 5CO_2 + I_2,$$
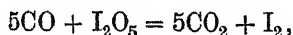
and either the carbon dioxide produced or the iodine liberated is measured.

Ethylene. This gas is rapidly absorbed by bromine water with formation of ethylene dibromide, $C_2H_4Br_2$. Water half saturated with bromine is most commonly used as the absorbent. Some chemists employ a solution of bromine in normal potassium bromide.

It should be noted that acetylene, although a more unsaturated compound than ethylene, reacts very slowly with bromine, and cannot therefore be determined in this way.

Nitric oxide. A saturated solution of ferrous sulphate containing a little free sulphuric acid may be used for absorbing this gas. Addition takes place with formation of nitrosoferrous sulphate, $[Fe(NO)]SO_4$. Alternatively the gas may be oxidized to nitrogen tetroxide, N_2O_4 or NO_2, by adding a known excess of oxygen. The nitrogen tetroxide is absorbed by potassium hydroxide and the unused oxygen by sodium hydrosulphite.

Certain other gases such as sulphur dioxide or hydrogen sulphide may be determined by absorption, but titration methods (see Chapter IV) are more suitable. Other gases are more conveniently determined by explosion as described later. Thus hydrogen may be absorbed by a solution of colloidal palladium, but is more con-

veniently estimated by explosion with oxygen. There is no satisfactory absorbent for nitrous oxide, so this gas is best determined by combustion with hydrogen.

It will be evident from the properties of the substances which are used for absorbing the various gases that some of them are capable of reacting with more than one gas. Thus an alkaline solution of sodium hydrosulphite will absorb carbon dioxide as well as oxygen. Ammoniacal cuprous chloride will absorb not only carbon monoxide but also acetylene and oxygen. It is therefore of fundamental importance to apply the reagents to a given gaseous mixture in the correct order. Thus if it is required to determine the proportions of the constituents of a gaseous mixture consisting of oxygen, nitrogen, carbon monoxide and carbon dioxide, a measured volume of the mixture is first treated with potash to remove carbon dioxide, secondly with sodium hydrosulphite to remove oxygen, and thirdly with cuprous chloride to remove carbon monoxide, nitrogen remaining unabsorbed, and is thus determined by difference.

Hempel's apparatus for gas analysis

A Hempel apparatus consists essentially of two parts, namely a gas burette for taking the volume measurements and a set of absorption bulbs which contain the various reagents for absorbing the gases. In Fig. 8 the measuring apparatus is shown connected with an absorption vessel. The tube A which contains a measured volume of the gaseous mixture is provided with a tap at the top, and is graduated in ml. divided into fifths throughout its entire length. Its total capacity is usually 100 ml. It is connected by rubber tubing to the pressure tube B. In making measurements the water, which is the confining liquid most generally used, is brought to the same level in both tubes so that the gas is measured at atmospheric pressure.

The simple absorption vessel C consists of two bulbs each of about 200 ml. capacity connected together by wide glass tubing so that the liquid may flow easily from one bulb into the other. Connexion with the measuring tube A is made from the capillary tubing of the attached to the upper part of the first bulb by a short length of stout rubber tubing provided with a screw clip attached
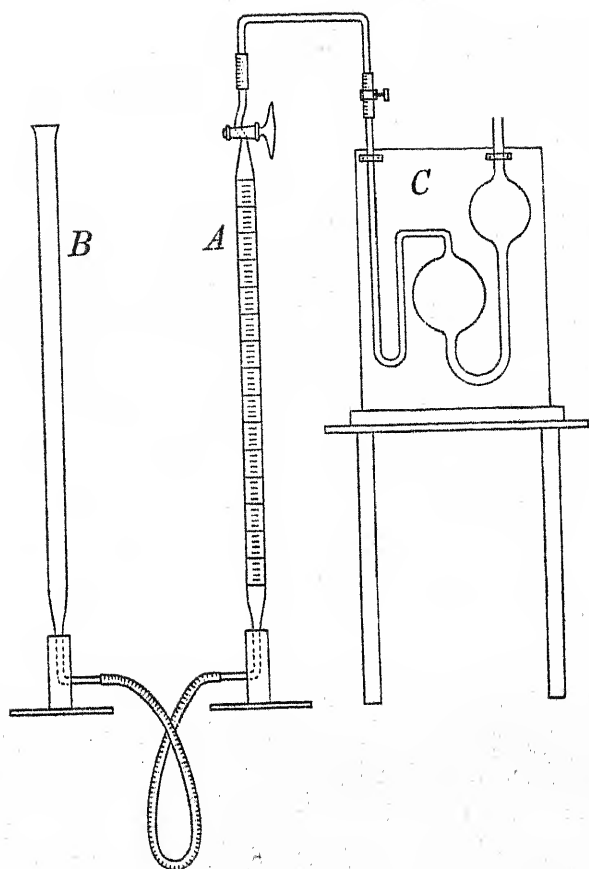
Fig. 8.
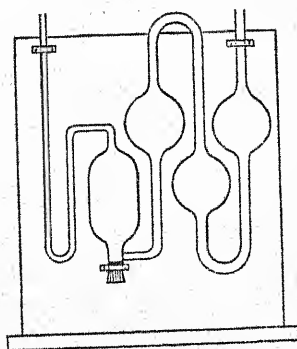
Fig. 9.

to a piece of capillary tubing bent twice at right angles which is attached by another short piece of stout rubber tubing to the tap on A. The volume of liquid absorbent is sufficient to fill one of the bulbs of C. In making an experiment, the liquid absorbent is drawn as far as a fixed point near the end of the capillary tubing attached to the bulb. The whole volume of gas in the measuring tube A is introduced into the bulb by opening the tap and the screw clip and raising the tube B. When the gas has been introduced the tap on the tube A and the screw clip are closed. The absorption bulbs are then removed and gently agitated for a short time to ensure thorough contact of the gas with the liquid reagent. The bulbs are again connected with the measuring apparatus, the screw clip and tap opened and the gas drawn back into the measuring tube A by lowering the pressure tube B. The tap is then closed and the water levels adjusted and the volume of gas measured. The process is then repeated with the same absorption bulb in order to ascertain whether absorption is complete. When the whole of the first gas has been removed from the mixture, the second set of absorption bulbs is brought into action to remove the second gas, then the third gas similarly absorbed and so on. In using reagents such as sodium hydrosulphite and ammoniacal cuprous chloride which are readily oxidized by exposure to the air, a set of double absorption bulbs one of which contains water in the bulb next but one adjacent to the bulb containing the reagent should be used. This enables the reagent to be protected from free exposure to the air. Fig. 9 shows a set of double bulbs of a type suitable for introducing a solid reagent.

The method of calculating the results may be illustrated with reference to the analysis of a mixture of oxygen, nitrogen, carbon monoxide and carbon dioxide with regard to which mention has been made of the correct order of absorbing the gases. Let v_1 be the volume in ml. of the original mixture. After treatment with potash this will become reduced to v_2 ml. The volume of carbon dioxide is clearly $(v_1 - v_2)$ ml. When the v_2 ml. which remain are treated with sodium hydrosulphite the volume will become reduced to v_3 ml. The volume of oxygen in the mixture is therefore $(v_2 - v_3)$ ml. Treatment of this last residue with ammoniacal cuprous chloride will reduce the volume to v_4 ml. which is the

volume of nitrogen, $(v_3 - v_4)$ ml. being the volume of carbon monoxide in the mixture.

Analysis of gases by explosion

Oxidizable gases, such as hydrogen, carbon monoxide, and hydrocarbons are estimated by explosion with oxygen. In some cases pure oxygen is used, or more usually combustion is effected with air. In any case, complete combustion must be secured by having excess of oxygen present. The quantity of combustible gas present is determined either from measurement of the oxygen which is consumed, or from the volume of the product of the reaction. If only one oxidizable gas is concerned, either measurement will suffice, but if two combustible gases are present, both measurements are necessary. A Hempel explosion pipette has two stout platinum wires fused into the upper part of the bulb and a tap at the bottom which connects with a reservoir so as to enable the water to be removed from the gaseous mixture when it is sparked. It is desirable to reduce the pressure of the gaseous mixture before sparking to avoid the risk of shattering the apparatus by the violence of the explosion.

When hydrogen is sparked with oxygen, two volumes of hydrogen react with one volume of oxygen, and the volume of liquid water produced at the ordinary temperature is negligible. If air is used, two volumes of hydrogen will require five volumes of air for complete oxidation, and will leave a residue of four volumes of nitrogen. Carbon monoxide requires half its own volume of oxygen for complete combustion, and produces its own volume of carbon dioxide. Hence carbon monoxide may be estimated either from the volume of oxygen which is consumed or from the volume of carbon dioxide which is produced. A consideration of the equations for the complete combustion of methane and ethylene,

$$CH_4 + 2O_2 = CO_2 + 2H_2O$$

and
$$C_2H_4 + 3O_2 = 2CO_2 + 2H_2O,$$
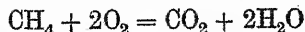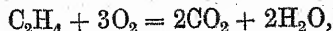
will show the volume relationships. It may be added that acetylene should never be determined by explosion, as on account of the endothermic character of the compound the explosion is

exceptionally violent. This gas may be estimated by absorption in a solution of ammoniacal silver chloride, prepared by saturating concentrated ammonia with freshly precipitated silver chloride. It should be noted that when a gaseous carbon compound having n atoms of carbon in the molecule is completely oxidized, the volume of carbon dioxide produced is always n times the volume of the gas taken for analysis.

In carrying out analyses by explosion, prolonged sparking must be avoided as otherwise some nitrogen may be oxidized to oxides of nitrogen, and serious errors would obviously arise as these products are absorbed by potash. Instead of effecting combination with oxygen by explosion, the analysis of oxidizable gases may also be effected by combustion with oxygen, using platinum or palladium as a catalyst. Combustion may also be effected with heated cupric oxide. It is even possible to adjust the conditions of combustion in such a manner as to effect *fractional* oxidation. Details for carrying out analyses of this kind are to be found in larger works.

The determination of carbon dioxide

Instead of determining carbon dioxide volumetrically by absorption in concentrated potassium hydroxide solution, this gas is very frequently determined gravimetrically by the same reaction, as is regularly practised in organic analysis. The analysis of carbonate rocks is effected by decomposition with hydrochloric acid, and the carbon dioxide thus evolved is purified from acid spray and moisture and passed into weighed potash bulbs. It should be added that the gravimetric analysis of carbonates should always be effected in this way, never by the use of an apparatus in which the carbonate is caused to react with the acid and the carbon dioxide measured by loss of weight. Results obtained in this way are at best only approximations.

For the determination of small quantities of carbon dioxide, such as occur in the atmosphere, other methods are employed. A titration method, originally due to Pettenkofer, consists in shaking a known volume of air in a stoppered bottle of at least two litres capacity together with a known quantity of standard barium hydroxide solution. The quantity of barium hydroxide should be at least three times the amount corresponding to the

carbon dioxide estimated to be present. After absorption has been judged to be complete, phenolphthalein is added and the excess of barium hydroxide determined by titration with standard acid. This method is not well suited to out-of-door work, and for such purposes a simpler and more convenient method devised by Lunge and Zenkendorff may be recommended. This consists in delivering known volumes of air by a special compression aspirator into a known volume of sodium carbonate solution ($N/500$) containing a little phenolphthalein. When a sufficient quantity of carbon dioxide has been absorbed by the solution to convert the whole of the sodium carbonate into bicarbonate, the colour of the phenolphthalein is discharged. The percentage of carbon dioxide in the air cannot, however, be calculated directly in this way, but a table has been drawn up by the authors showing the relation between the number of bulb fillings and the percentage of carbon dioxide in the air (see Lunge and Ambler, *Technical Gas Analysis*, p. 155).

The experimental study of gases is receiving much attention at the present time, and in view of the rapidly increasing use of gaseous reactions in manufacturing processes, the subject of gas analysis is almost certain to assume greater importance. For further study of this subject the reader may be referred to Lunge's *Technical Gas Analysis*, revised by Ambler, 1934, and to Sutton's *Volumetric Analysis*, Twelfth Edition, revised by A. D. Mitchell, 1935, Part x (revised by J. S. G. Thomas, 1934).

Atomic Weights (O = 16)

	Symbol	At. No.	At. wt.		Symbol	At. No.	At. wt.
Aluminium	Al	13	26·97	Neon	Ne	10	20·183
Antimony	Sb	51	121·76	Nickel	Ni	28	58·69
Argon	A	18	39·944	Niobium	Nb (Cb)	41	92·91
Arsenic	As	33	74·91	(Columbium)			
Barium	Ba	56	137·36	Nitrogen	N	7	14·008
Beryllium	Be	4	9·02	Osmium	Os	76	191·5
Bismuth	Bi	83	209·00	Oxygen	O	8	16·0000
Boron	B	5	10·82	Palladium	Pd	46	106·7
Bromine	Br	35	79·916	Phosphorus	P	15	31·02
Cadmium	Cd	48	112·41	Platinum	Pt	78	195·23
Caesium	Cs	55	132·91	Potassium	K	19	39·096
Calcium	Ca	20	40·08	Praseodymium	Pr	59	140·92
Carbon	C	6	12·01	Protoactinium	Pa	91	231
Cerium	Ce	58	140·13	Radium	Ra	88	226·05
Chlorine	Cl	17	35·457	Radon	Rn	86	222
Chromium	Cr	24	52·01	Rhenium	Re	75	186·31
Cobalt	Co	27	58·94	Rhodium	Rh	45	102·91
Copper	Cu	29	63·57	Rubidium	Rb	37	85·48
Dysprosium	Dy	66	162·46	Ruthenium	Ru	44	101·7
Erbium	Er	68	167·64	Samarium	Sm	62	150·43
Europium	Eu	63	152·0	Scandium	Sc	21	45·10
Fluorine	F	9	19·00	Selenium	Se	34	78·96
Gadolinium	Gd	64	156·9	Silicon	Si	14	28·06
Gallium	Ga	31	69·72	Silver	Ag	47	107·880
Germanium	Ge	32	72·60	Sodium	Na	11	22·997
Gold	Au	79	197·2	Strontium	Sr	38	87·63
Hafnium	Hf	72	178·6	Sulphur	S	16	32·06
Helium	He	2	4·002	Tantalum	Ta	73	180·88
Holmium	Ho	67	163·5	Tellurium	Te	52	127·61
Hydrogen	H	1	1·0078	Terbium	Tb	65	159·2
Indium	In	49	114·76	Thallium	Tl	81	204·39
Iodine	I	53	126·92	Thorium	Th	90	232·12
Iridium	Ir	77	193·1	Thulium	Tm	69	169·4
Iron	Fe	26	55·84	Tin	Sn	50	118·70
Krypton	Kr	36	83·7	Titanium	Ti	22	47·90
Lanthanum	La	57	138·92	Tungsten	W	74	184·0
Lead	Pb	82	207·21	Uranium	U	92	238·07
Lithium	Li	3	6·940	Vanadium	V	23	50·95
Lutecium	Lu	71	175·0	Xenon	Xe	54	131·3
Magnesium	Mg	12	24·32	Ytterbium	Yb	70	173·04
Manganese	Mn	25	54·93	Yttrium	Y	39	88·92
Mercury	Hg	80	200·61	Zinc	Zn	30	65·38
Molybdenum	Mo	42	96·0	Zirconium	Zr	40	91·22
Neodymium	Nd	60	144·27				

Formula Values of Certain Substances ($O = 16$)

Acetic acid, CH_3COOH, 60·03
Acetaldehyde, CH_3CHO, 44·03
Ammonia, NH_3, 17·034
Ammonium chloride, NH_4Cl, 53·5
Ammonium thiocyanate, NH_4CNS, 76·12
Arsenious oxide, As_2O_3, 197·92

Barium sulphate, $BaSO_4$, 233·4
Borax, $Na_2B_4O_7 10H_2O$, 382·16

Calcium carbonate, $CaCO_3$, 100·07
Citric acid, CH_2COOH

$$C(OH)COOH + H_2O, \ 210\cdot08$$

CH_2COOH
Cupric sulphate, $CuSO_4 5H_2O$, 249·67
Cuprous thiocyanate, $CuCNS$, 121·6

Ferric oxide, Fe_2O_3, 159·6
Ferrous ammonium sulphate, $FeSO_4$ $(NH_4)_2SO_4 6H_2O$, 392·17. This substance contains one-seventh of its own weight of iron
Formaldehyde, $HCHO$, 30·02

Hydrochloric acid, HCl, 36·47
Hydrogen peroxide, H_2O_2, 34·016
Hydrogen sulphide, H_2S, 34·09

Lead sulphate, $PbSO_4$, 303·2

Magnesium pyrophosphate, $Mg_2P_2O_7$, 222·6
Manganese pyrophosphate, $Mn_2P_2O_7$, 284

Nickel dimethylglyoxime, $C_8H_{14}N_4O_4Ni$, 288·7
Nitric acid, HNO_3, 63·02

Orthophosphoric acid, H_3PO_4, 98·064
Oxalic acid, $COOH$

$$|, \ 2H_2O, \ 126\cdot05$$
$COOH$

Phosphomolybdic anhydride, P_2O_5, $24MoO_3$, 3598
Potassium bromide, KBr, 119·03
Potassium chloride, KCl, 74·56
Potassium cyanide, KCN, 65·11
Potassium dichromate, $K_2Cr_2O_7$, 294·2
Potassium ferricyanide, $K_3Fe(CN)_6$, 329·1
Potassium ferrocyanide, $K_4Fe(CN)_6 3H_2O$, 422·36
Potassium hydroxide, KOH, 56·1
Potassium iodate, KIO_3, 214·0
Potassium iodide, KI, 166·02
Potassium permanganate, $KMnO_4$, 158·03

Silver chloride, $AgCl$, 143·3
Silver nitrate, $AgNO_3$, 169·89
Sodium bicarbonate, $NaHCO_3$, 84·01
Sodium carbonate (anhydrous), Na_2CO_3, 106
Sodium carbonate, decahydrate, $Na_2CO_3 10H_2O$, 286·16
Sodium chloride, $NaCl$, 58·46
Sodium hydroxide, $NaOH$, 40·01
Sodium oxalate, $Na_2C_2O_4$, 134
Sodium thiosulphate, $Na_2S_2O_3 5H_2O$, 248·22
Succinic acid, CH_2COOH, 118·05

CH_2COOH
Sulphur dioxide, SO_2, 64·07
Sulphuric acid, H_2SO_4, 98·086

Tartaric acid, $CH(OH)COOH$, 150·05

$CH(OH)COOH$

Water, H_2O, 18·016 $7H_2O$, 126·11
$2H_2O$, 36·032 $8H_2O$, 144·13
$3H_2O$, 54·048 $9H_2O$, 162·14
$4H_2O$, 72·064 $10H_2O$, 180·16
$5H_2O$, 90·08 $11H_2O$, 198·18
$6H_2O$, 108·10 $12H_2O$, 216·19

Zinc sulphate, $ZnSO_4 7H_2O$, 287·55

Pressure of Aqueous Vapour in mm. of Mercury

Temp. °C.	Pressure mm.	Temp. °C.	Pressure mm.	Temp. °C.	Pressure mm.
5	6·53	12	10·46	19	16·35
6	7·00	13	11·16	20	17·39
7	7·49	14	11·91	21	18·50
8	8·02	15	12·70	22	19·66
9	8·57	16	13·54	23	20·89
10	9·17	17	14·42	24	22·18
11	9·79	18	15·36	25	23·55

Table of Hardness, Parts in 100,000

Column I denotes the number of c.c. of standard soap solution, and Column II the amount of $CaCO_3$ per 100,000

I	II	I	II	I	II	I	II	I	II
0·7	0·00	3·8	4·29	6·9	8·71	10·0	13·31	13·1	18·17
0·8	·16	·9	·43	7·0	·86	·1	·46	·2	·33
0·9	·32	4·0	·57	·1	9·00	·2	·61	·3	·49
1·0	·48	·1	·71	·2	·14	·3	·76	·4	·65
·1	·63	·2	·86	·3	·29	·4	·91	·5	·81
·2	·79	·3	5·00	·4	·43	·5	14·06	·6	·97
·3	·95	·4	·14	·5	·57	·6	·21	·7	19·13
·4	1·11	·5	·29	·6	·71	·7	·37	·8	·29
·5	·27	·6	·43	·7	·86	·8	·52	·9	·44
·6	·43	·7	·57	·8	10·00	·9	·68	14·0	·60
·7	·56	·8	·71	·9	·15	11·0	·84	·1	·76
·8	·69	·9	·86	8·0	·30	·1	15·00	·2	·92
·9	·82	5·0	6·00	·1	·45	·2	·16	·3	20·08
2·0	·95	·1	·14	·2	·60	·3	·32	·4	·24
·1	2·08	·2	·29	·3	·75	·4	·48	·5	·40
·2	·21	·3	·43	·4	·90	·5	·63	·6	·56
·3	·34	·4	·57	·5	11·05	·6	·79	·7	·71
·4	·47	·5	·71	·6	·20	·7	·95	·8	·87
·5	·60	·6	·86	·7	·35	·8	16·11	·9	21·03
·6	·73	·7	7·00	·8	·50	·9	·27	15·0	·19
·7	·86	·8	·14	·9	·65	12·0	·43	·1	·35
·8	·99	·9	·29	9·0	·80	·1	·59	·2	·51
·9	3·12	6·0	·43	·1	·95	·2	·75	·3	·68
3·0	·25	·1	·57	·2	12·11	·3	·90	·4	·85
·1	·38	·2	·71	·3	·26	·4	17·06	·5	22·02
·2	·51	·3	·86	·4	·41	·5	·22	·6	·18
·3	·64	·4	8·00	·5	·56	·6	·38	·7	·35
·4	·77	·5	·14	·6	·71	·7	·54	·8	·52
·5	·90	·6	·29	·7	·86	·8	·70	·9	·69
·6	4·03	·7	·43	·8	13·01	·9	·86	16·0	·86
·7	·16	·8	·57	·9	·16	13·0	18·02		

Relation between specific gravity and percentage composition of solutions of hydrochloric acid (in grammes of solute to 100 grammes of solution)

(Lunge and Marchlewski)

d_4^{15}	%	d_4^{15}	%	d_4^{15}	%
1·000	0·16	1·070	14·17	1·140	27·66
1·005	1·15	1·075	15·16	1·145	28·61
1·010	2·14	1·080	16·15	1·150	29·57
1·015	3·12	1·085	17·13	1·155	30·55
1·020	4·13	1·090	18·11	1·160	31·52
1·025	5·15	1·095	19·06	1·165	32·49
1·030	6·15	1·100	20·01	1·170	33·46
1·035	7·15	1·105	20·97	1·175	34·42
1·040	8·16	1·110	21·92	1·180	35·39
1·045	9·16	1·115	22·86	1·185	36·31
1·050	10·17	1·120	23·82	1·190	37·23
1·055	11·18	1·125	24·78	1·195	38·16
1·060	12·19	1·130	25·75	1·200	39·11
1·065	13·19	1·135	26·70		

Relation between specific gravity and percentage composition of solutions of sodium hydroxide (in grammes of solute to 100 grammes of solution)

Bousfield and Lowry (*Phil. Trans.*, 1905, 204 A, p. 253)

d_4^{15}	%	d_4^{15}	%	d_4^{15}	%
0·99918	0	1·18868	17	1·3728	34
1·01065	1	1·19973	18	1·3830	35
1·02198	2	1·21079	19	1·3933	36
1·03322	3	1·22183	20	1·4034	37
1·04441	4	1·23285	21	1·4135	38
1·05554	5	1·24386	22	1·4235	39
1·06666	6	1·25485	23	1·4334	40
1·07777	7	1·26582	24	1·4432	41
1·08887	8	1·27679	25	1·4529	42
1·09997	9	1·2877	26	1·4625	43
1·11107	10	1·2986	27	1·4720	44
1·12217	11	1·3094	28	1·4815	45
1·13327	12	1·3202	29	1·4911	46
1·14436	13	1·3309	30	1·5007	47
1·15545	14	1·3415	31	1·5102	48
1·16653	15	1·3520	32	1·5196	49
1·17761	16	1·3624	33	1·5290	50

LOGARITHMS.

	0	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
10	0000	0043	0086	0128	0170	0212	0253	0294	0334	0374	4	8	12	17	21	25	29	33	37
11	0414	0453	0492	0531	0569	0607	0645	0682	0719	0755	4	8	11	15	19	23	26	30	34
12	0792	0828	0864	0899	0934	0969	1004	1038	1072	1106	3	7	10	14	17	21	24	28	31
13	1139	1173	1206	1239	1271	1303	1335	1367	1399	1430	3	6	10	13	16	19	23	26	29
14	1461	1492	1523	1553	1584	1614	1644	1673	1703	1732	3	6	9	12	15	18	21	24	27
15	1761	1790	1818	1847	1875	1903	1931	1959	1987	2014	3	6	8	11	14	17	20	22	25
16	2041	2068	2095	2122	2148	2175	2201	2227	2253	2279	3	5	8	11	13	16	18	21	24
17	2304	2330	2355	2380	2405	2430	2455	2480	2504	2529	2	5	7	10	12	15	17	20	22
18	2553	2577	2601	2625	2648	2672	2695	2718	2742	2765	2	5	7	9	12	14	16	19	21
19	2788	2810	2833	2856	2878	2900	2923	2945	2967	2989	2	4	7	9	11	13	16	18	20
20	3010	3032	3054	3075	3096	3118	3139	3160	3181	3201	2	4	6	8	11	13	15	17	19
21	3222	3243	3263	3284	3304	3324	3345	3365	3385	3404	2	4	6	8	10	12	14	16	18
22	3424	3444	3464	3483	3502	3522	3541	3560	3579	3598	2	4	6	8	10	12	14	15	17
23	3617	3636	3655	3674	3692	3711	3729	3747	3766	3784	2	4	6	7	9	11	13	15	17
24	3802	3820	3838	3856	3874	3892	3909	3927	3945	3962	2	4	5	7	9	11	12	14	16
25	3979	3997	4014	4031	4048	4065	4082	4099	4116	4133	2	3	5	7	9	10	12	14	15
26	4150	4166	4183	4200	4216	4232	4249	4265	4281	4298	2	3	5	7	8	10	11	13	15
27	4314	4330	4346	4362	4378	4393	4409	4425	4440	4456	2	3	5	6	8	9	11	13	14
28	4472	4487	4502	4518	4533	4548	4564	4579	4594	4609	2	3	5	6	8	9	11	12	14
29	4624	4639	4654	4669	4683	4698	4713	4728	4742	4757	1	3	4	6	7	9	10	12	13
30	4771	4786	4800	4814	4829	4843	4857	4871	4886	4900	1	3	4	6	7	9	10	11	13
31	4914	4928	4942	4955	4969	4983	4997	5011	5024	5038	1	3	4	6	7	8	10	11	12
32	5051	5065	5079	5092	5105	5119	5132	5145	5159	5172	1	3	4	5	7	8	9	11	12
33	5185	5198	5211	5224	5237	5250	5263	5276	5289	5302	1	3	4	5	6	8	9	10	12
34	5315	5328	5340	5353	5366	5378	5391	5403	5416	5428	1	3	4	5	6	8	9	10	11
35	5441	5453	5465	5478	5490	5502	5514	5527	5539	5551	1	2	4	5	6	7	9	10	11
36	5563	5575	5587	5599	5611	5623	5635	5647	5658	5670	1	2	4	5	6	7	8	10	11
37	5682	5694	5705	5717	5729	5740	5752	5763	5775	5786	1	2	3	5	6	7	8	9	10
38	5798	5809	5821	5832	5843	5855	5866	5877	5888	5899	1	2	3	5	6	7	8	9	10
39	5911	5922	5933	5944	5955	5966	5977	5988	5999	6010	1	2	3	4	5	7	8	9	10
40	6021	6031	6042	6053	6064	6075	6085	6096	6107	6117	1	2	3	4	5	6	8	9	10
41	6128	6138	6149	6160	6170	6180	6191	6201	6212	6222	1	2	3	4	5	6	7	8	9
42	6232	6243	6253	6263	6274	6284	6294	6304	6314	6325	1	2	3	4	5	6	7	8	9
43	6335	6345	6355	6365	6375	6385	6395	6405	6415	6425	1	2	3	4	5	6	7	8	9
44	6435	6444	6454	6464	6474	6484	6493	6503	6513	6522	1	2	3	4	5	6	7	8	9
45	6532	6542	6551	6561	6571	6580	6590	6599	6609	6618	1	2	3	4	5	6	7	8	9
46	6628	6637	6646	6656	6665	6675	6684	6693	6702	6712	1	2	3	4	5	6	7	7	8
47	6721	6730	6739	6749	6758	6767	6776	6785	6794	6803	1	2	3	4	4	5	6	7	8
48	6812	6821	6830	6839	6848	6857	6866	6875	6884	6893	1	2	3	4	4	5	6	7	8
49	6902	6911	6920	6928	6937	6946	6955	6964	6972	6981	1	2	3	4	4	5	6	7	8
50	6990	6998	7007	7016	7024	7033	7042	7050	7059	7067	1	2	3	3	4	5	6	7	8
51	7076	7084	7093	7101	7110	7118	7126	7135	7143	7152	1	2	3	3	4	5	6	7	8
52	7160	7168	7177	7185	7193	7202	7210	7218	7226	7235	1	2	2	3	4	5	6	7	7
53	7243	7251	7259	7267	7275	7284	7292	7300	7308	7316	1	2	2	3	4	5	6	6	7
54	7324	7332	7340	7348	7356	7364	7372	7380	7388	7396	1	2	2	3	4	5	6	6	7

	0	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
55	·7404	7412	7419	7427	7435	7443	7451	7459	7466	7474	1	2	2	3	4	5	5	6	7
56	·7482	7490	7497	7505	7513	7520	7528	7536	7543	7551	1	2	2	3	4	5	5	6	7
57	·7559	7566	7574	7582	7589	7597	7604	7612	7619	7627	1	2	2	3	4	5	5	6	7
58	·7634	7642	7649	7657	7664	7672	7679	7686	7694	7701	1	1	2	3	4	5	5	6	7
59	·7709	7716	7723	7731	7738	7745	7752	7760	7767	7774	1	1	2	3	4	4	5	6	7
60	·7782	7789	7796	7803	7810	7818	7825	7832	7839	7846	1	1	2	3	4	4	5	6	6
61	·7853	7860	7868	7875	7882	7889	7896	7903	7910	7917	1	1	2	3	4	4	5	6	6
62	·7924	7931	7938	7945	7952	7959	7966	7973	7980	7987	1	1	2	3	3	4	5	6	6
63	·7993	8000	8007	8014	8021	8028	8035	8041	8048	8055	1	1	2	3	3	4	5	5	6
64	·8062	8069	8075	8082	8089	8096	8102	8109	8116	8122	1	1	2	3	3	4	5	5	6
65	·8129	8136	8142	8149	8156	8162	8169	8176	8182	8189	1	1	2	3	3	4	5	5	6
66	·8195	8202	8209	8215	8222	8228	8235	8241	8248	8254	1	1	2	3	3	4	5	5	6
67	·8261	8267	8274	8280	8287	8293	8299	8306	8312	8319	1	1	2	3	3	4	5	5	6
68	·8325	8331	8338	8344	8351	8357	8363	8370	8376	8382	1	1	2	3	3	4	4	5	6
69	·8388	8395	8401	8407	8414	8420	8426	8432	8439	8445	1	1	2	2	3	4	4	5	6
70	·8451	8457	8463	8470	8476	8482	8488	8494	8500	8506	1	1	2	2	3	4	4	5	6
71	·8513	8519	8525	8531	8537	8543	8549	8555	8561	8567	1	1	2	2	3	4	4	5	5
72	·8573	8579	8585	8591	8597	8603	8609	8615	8621	8627	1	1	2	2	3	4	4	5	5
73	·8633	8639	8645	8651	8657	8663	8669	8675	8681	8686	1	1	2	2	3	4	4	5	5
74	·8692	8698	8704	8710	8716	8722	8727	8733	8739	8745	1	1	2	2	3	4	4	5	5
75	·8751	8756	8762	8768	8774	8779	8785	8791	8797	8802	1	1	2	2	3	3	4	5	5
76	·8808	8814	8820	8825	8831	8837	8842	8848	8854	8859	1	1	2	2	3	3	4	5	5
77	·8865	8871	8876	8882	8887	8893	8899	8904	8910	8915	1	1	2	2	3	3	4	4	5
78	·8921	8927	8932	8938	8943	8949	8954	8960	8965	8971	1	1	2	2	3	3	4	4	5
79	·8976	8982	8987	8993	8998	9004	9009	9015	9020	9025	1	1	2	2	3	3	4	4	5
80	·9031	9036	9042	9047	9053	9058	9063	9069	9074	9079	1	1	2	2	3	3	4	4	5
81	·9085	9090	9096	9101	9106	9112	9117	9122	9128	9133	1	1	2	2	3	3	4	4	5
82	·9138	9143	9149	9154	9159	9165	9170	9175	9180	9186	1	1	2	2	3	3	4	4	5
83	·9191	9196	9201	9206	9212	9217	9222	9227	9232	9238	1	1	2	2	3	3	4	4	5
84	·9243	9248	9253	9258	9263	9269	9274	9279	9284	9289	1	1	2	2	3	3	4	4	5
85	·9294	9299	9304	9309	9315	9320	9325	9330	9335	9340	1	1	2	2	3	3	4	4	5
86	·9345	9350	9355	9360	9365	9370	9375	9380	9385	9390	1	1	1	2	3	3	4	4	5
87	·9395	9400	9405	9410	9415	9420	9425	9430	9435	9440	0	1	1	2	2	3	3	4	4
88	·9445	9450	9455	9460	9465	9469	9474	9479	9484	9489	0	1	1	2	2	3	3	4	4
89	·9494	9499	9504	9509	9513	9518	9523	9528	9533	9538	0	1	1	2	2	3	3	4	4
90	·9542	9547	9552	9557	9562	9566	9571	9576	9581	9586	0	1	1	2	2	3	3	4	4
91	·9590	9595	9600	9605	9609	9614	9619	9624	9628	9633	0	1	1	2	2	3	3	4	4
92	·9638	9643	9647	9652	9657	9661	9666	9671	9675	9680	0	1	1	2	2	3	3	4	4
93	·9685	9689	9694	9699	9703	9708	9713	9717	9722	9727	0	1	1	2	2	3	3	4	4
94	·9731	9736	9741	9745	9750	9754	9759	9763	9768	9773	0	1	1	2	2	3	3	4	4
95	·9777	9782	9786	9791	9795	9800	9805	9809	9814	9818	0	1	1	2	2	3	3	4	4
96	·9823	9827	9832	9836	9841	9845	9850	9854	9859	9863	0	1	1	2	2	3	3	4	4
97	·9868	9872	9877	9881	9886	9890	9894	9899	9903	9908	0	1	1	2	2	3	3	4	4
98	·9912	9917	9921	9926	9930	9934	9939	9943	9948	9952	0	1	1	2	2	3	3	4	4
99	·9956	9961	9965	9969	9974	9978	9983	9987	9991	9996	0	1	1	2	2	3	3	3	4

ANTI-LOGARITHMS.

	0	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
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D. = Determination

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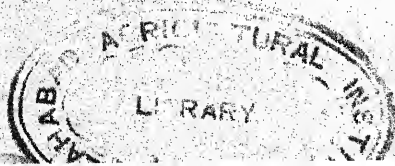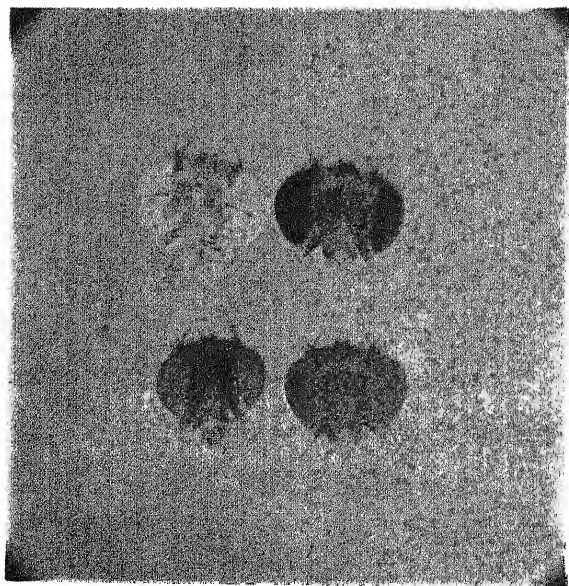
Frontispiece. Eye colours of the fruit fly, *Drosophila melanogaster: top left*, white eye (w); *top right*, sepia (se); *bottom left*, eosin (wᵉ); *bottom right*, wild type (+). (Photograph courtesy of Mr. P. F. Hulley.)

AN INTRODUCTION TO

INSECT PHYSIOLOGY

by

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PREFACE

The need for another textbook on insect physiology may not seem particularly compelling, as students of this subject are already well served by a number of outstanding texts. These include several excellent review series like *Advances in Insect Physiology* and *Annual Review of Entomology*; a number of good co-operative enterprises among which "Insect Physiology", edited by K. Roeder, and "The Physiology of Insecta", edited by M. Rockstein, may be mentioned; and, bright among these lesser lights, successive editions of Wigglesworth's unrivalled "The Principles of Insect Physiology". It is my experience as a teacher, however, that these books provide too heavy a fare for undergraduates who are not yet fully committed to the study of insect physiology, and it is for such students that the present volume is intended. It attempts to provide a less detailed account of a field which continues to be fruitful for the elucidation of many fundamental aspects of physiological function; and it is written in the hope that a broader approach may better serve to quicken the interest of potential converts.

In order to present a general treatment of insect physiology in a book of manageable size, it has been necessary to assume a certain knowledge of physiology on the part of the reader. Against this assumed background, those aspects of insect physiology which appear to be characteristic of the class have been singled out for detailed discussion. Attempts have been made, wherever possible, to sketch in the experimental background to the information which is presented, by coupling the account of a given topic with appropriate illustrations from the research literature. To these illustrations full reference is provided, giving a point of entry into the literature for readers who may wish to go more deeply into a particular subject; the text itself has been kept free of references, except where it has been thought desirable to draw attention to important work not included among the text figures.

The common names of insects, like cockroach or blowfly, are often used in the text where there seems little to be gained by a more precise designation. This practice does raise the difficulty that there are a number of quite different types of blowfly or cockroach, and what is said of one type does not necessarily apply to another. To avoid possible misinterpretation, an appendix is provided that sets out the common names of insects mentioned in the text, and lists the scientific names of species of that type which have been used as experimental material, together with a page reference to the corresponding item of information.

August, 1970   E. BURSELL
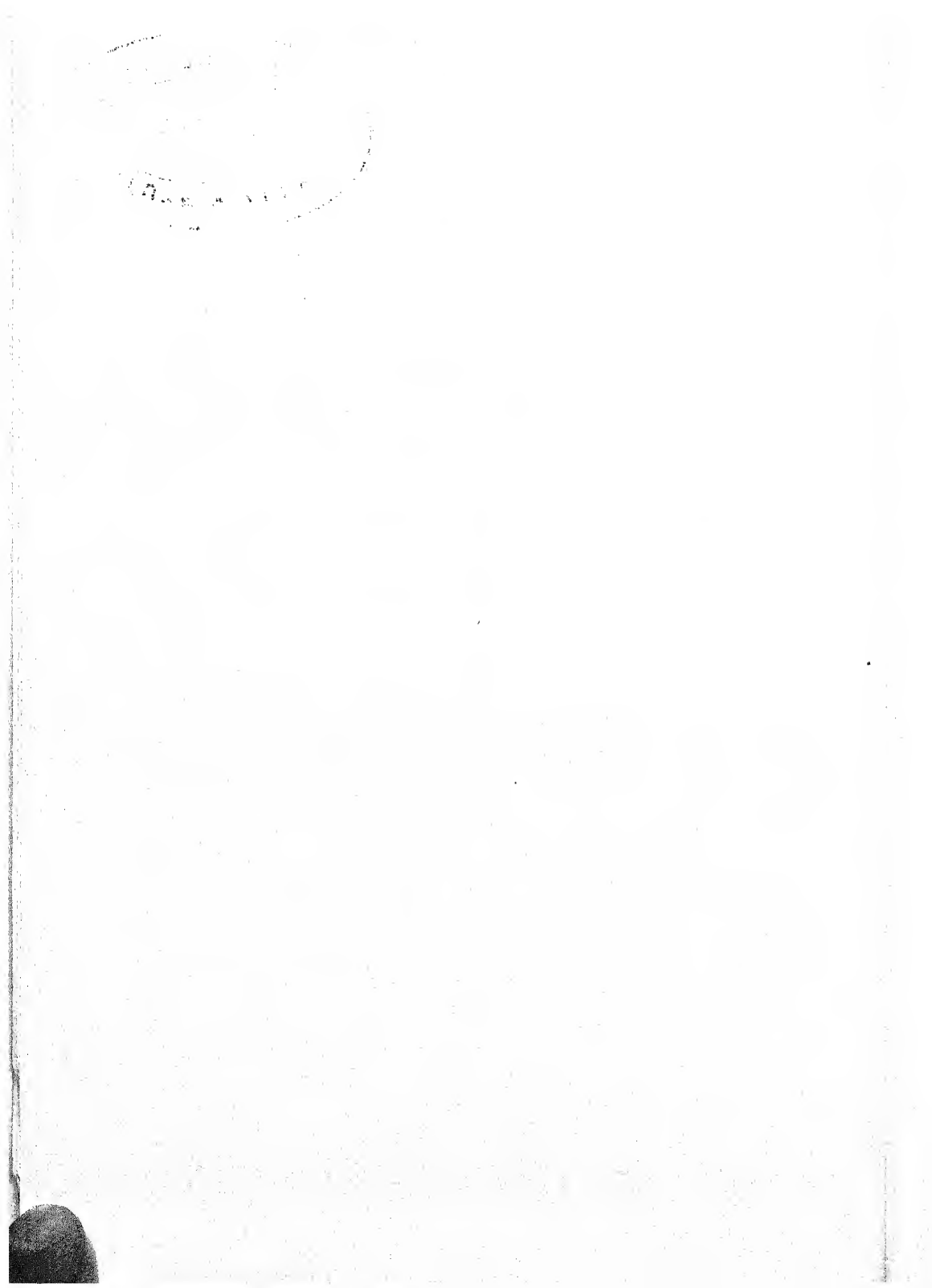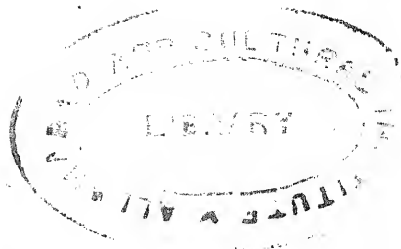
INTRODUCTION

Before setting out to discuss the physiology of insects it may be useful to attempt some definition of what, in the present context, will be meant by the word "physiology", and what by the word "insect", and in this way to delimit the general field of enquiry.

The approach to physiology which I propose to adopt is based on the view that insects, like any other form of animal life, can ultimately be regarded as self-replicating metabolic systems; systems, that is, which possess the catalysts and cofactors necessary to promote a particular pattern of transformation of energy and material, to sustain a particular type of metabolism. Usually such a pattern involves the breakdown of complex organic molecules, with capture of a part of the energy so released in useful form; and the synthesis, usually from simpler molecules, of components of the metabolic system itself. In order that such a system shall continue to exist a number of requirements must be fulfilled. There must, for instance, be a continual supply of complex molecules to serve as a source of energy and as raw material for synthetic purposes; oxygen must be supplied to meet the needs of oxidative degradations; the catalytic machinery must be maintained in an environment suitable for its activity, necessitating the removal of toxic end products and the regulation of water content and ionic composition. The term somatic physiology may be used to denote the processes by which these different requirements are met. It would include, for example, the processes of nutrition, digestion, respiratory exchange, excretion and osmoregulation, studied largely at the level of organ systems, such as the alimentary canal, the tracheal system and the excretory system. In addition there are activities of the organism as a whole which tend to the fulfilment of metabolic requirements, and here one would be concerned with a study of the behaviour of the organism in relation to its environment, as mediated by the neuromuscular system. Finally, there are those aspects of physiology which relate particularly to the self-replicating nature of the system, and these may be considered under the separate heading of reproduction and development. Genetical aspects of replication have, somewhat arbitrarily, been deemed to lie outside the scope of the present book.

In what follows, the word "physiology" will thus be used to denote the sum total of processes tending to the maintenance and replication of a metabolic system. And this book aims to deal with that kind of metabolic system which is called an insect. There are, however, over a million species of insect, and between them they show an amazing diversity of adaptation to widely different

modes of life, many of them involving profound specializations at the physiological level. One could therefore envisage the existence of a large number of types of insect physiology, and in a general treatment some sort of simplification would be essential. The question arises as to the basis on which such simplification could reasonably be made. One way would be to limit consideration to what one might hopefully regard as a "typical" insect. But apart from the difficulty of deciding what kind of animal this would be, one is faced with the fact that our knowledge of insect physiology is somewhat fragmentary. Certain aspects of physiology are well documented for certain insects, others for others, but in no single species are we in a position to build up anything like a complete picture. In view of this, a better approach might be to limit consideration as far as possible to those aspects of described physiology which could be thought to be typical of insects generally, rather than just of a particular species. Here an element of subjective judgement is involved, but since simplification must inevitably mean selection of some kind, this cannot be avoided. What can be done, however, is to elaborate a little on the sort of criteria which could be used as a basis for selection; to consider, in other words, what are the characteristics of insects generally which would be most likely to affect their physiology. Here one would perhaps list, in the first place their terrestrialness, with all this implies in terms of desiccation, insolation, thermal fluctuations and so on. There is, secondly, their smallness; the largest insects are barely as big as the smallest terrestrial vertebrates, while the smallest insects are little bigger than many protozoans. This means that the surfaces available for exchanges with the environment are large in relation to the volume which serves as the source or sink of the exchange. This surface/volume relationship has wide implications in relation to such aspects as water balance, heat balance and respiratory exchange. The capacity for flight is another characteristic of insects which could be expected to have far-reaching physiological implications, with particular reference to neuromuscular physiology and to the mobilization of metabolic reserves. Fourthly, and perhaps most importantly, there is the fact that insects are exoskeletal; that their cuticle must play a dual role, as a skeleton and as a protective layer. This is a feature of very great consequence, particularly in connection with growth and development, and with aspects of metabolism associated with the deposition of the cuticle.

It would be possible to extend this list very considerably, but enough has perhaps been said to indicate the sorts of features which seem likely particularly to affect insect physiology, and which have therefore hopefully been used as a guide in deciding what is relevant to a general discussion of insect physiology, as against what may be regarded as a peculiar characteristic of this or that particular species of insect.

ACKNOWLEDGEMENTS

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SECTION I: SOMATIC PHYSIOLOGY

SECTION II: NEUROMUSCULAR PHYSIOLOGY

Somatic Physiology

METABOLISM

Metabolism may be considered as being of two main kinds; one involving the breakdown of complex organic molecules and the trapping of part of the energy which they contain in high energy phosphate linkage; the other involving a synthesis of complex organic molecules from simpler ones, to replace or renew components of the metabolic system. The two kinds have many enzymes and pathways in common, and are not to be considered as distinct entities, but they do differ in general direction and may conveniently be separated for purposes of discussion.

1. The Breakdown of Complex Organic Molecules

Three main classes of compounds, carbohydrates, proteins and fats are used as sources of energy in insects, as in most other animals. A general outline of the main pathways, showing the interrelationships between the three classes is illustrated in Fig. 1.1, which will serve as a framework for the more detailed discussion which follows.

The focal point of degradation metabolism is the Krebs cycle. This is a complicated system of enzymes, co-factors and substrates which effects the complete oxidation of 2-carbon fragments to carbon dioxide and water. The operation is achieved through a series of decarboxylations and dehydrogenations. The carbon dioxide is released into solution, while the hydrogen which is freed from substrate combination is handed down through a series of hydrogen or electron carriers, of which nicotine adenine dinucleotide (NAD), the flavoproteins and the cytochromes are familiar examples. The electrons are eventually passed on to molecular oxygen with the formation of water, as shown at the bottom of Fig. 1.1. This transport of hydrogen is coupled with a process of phosphorylation, so that a substantial proportion of the energy released in the process of dehydrogenation is captured in high energy phosphate linkage ($\sim$P) leading to the formation of adenosine triphosphate (ATP) from adenosine diphosphate (ADP). The high energy of the terminal phosphate radicle represents what may be called usable energy, and $\sim$P constitutes the common

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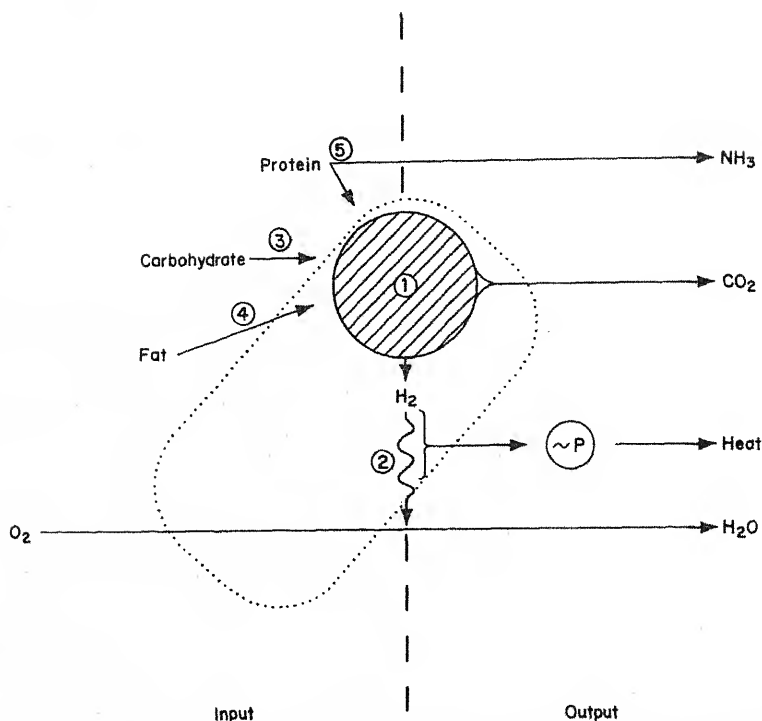
Fig. 1.1. An outline of metabolism. 1, Krebs cycle; 2, hydrogen transport; 3, glycolysis; 4, β-oxidation; 5, deamination; the dotted line delimits mitochondrial events; for further explanation see text.

coin of energy expenditure, being used in virtually all of the energy demanding processes requisite to the maintenance of the metabolic system as a whole, to the maintenance, that is, of the living state. Thus ATP is required for the contraction of muscle, for a variety of chemical syntheses, for the performance of osmotic work, for the production of light in luminescent organs, and so on. The precise way in which the energy of the phosphate bond is transformed into various other forms of energy is still largely unknown, but for present purposes it is enough to accept that the production of ATP, which occurs mainly as a result of oxidative phosphorylation, is an indispensable condition of life.

The input to this central part of the metabolic system is oxygen and foodstuff, as shown on the left side of Fig. 1.1. Carbohydrates are degraded to a form suitable for entry into the Krebs cycle in the process known as glycolysis. This constitutes a fragmentation of the 6-carbon molecule, which arises by hydrolysis of polysaccharides like glycogen, and involves initial phosphorylations, which serve to bring the molecule into a reactive state, and so facilitate the splitting into two 3-carbon fragments. Each step in the process is catalysed

by its own enzyme, as is the case in nearly all of the metabolic transformations under discussion. After further modification the 3-carbon fragment undergoes a process of oxidative decarboxylation in combination with coenzyme A (CoA), to yield the 2-carbon fragment, still linked to the enzyme as acetyl CoA; and it is in this form that it enters the Krebs cycle, to become involved in the process of oxidative phosphorylation. Except for the final decarboxylation, the breakdown of glucose to its 2-carbon derivative takes place in the absence of oxygen, i.e. it is anaerobic.

The fats undergo preliminary hydrolysis to yield their constituent parts, glycerol and fatty acids. Glycerol is phosphorylated to join the glycolytic pathway, while the fatty acids are subjected to a process known as β-oxidation. This involves the splitting off of successive 2-carbon fragments, which takes place in combination with CoA to yield successive molecules of acetyl CoA, of which the 2-carbon fragment enters the Krebs cycle.

The proteins are hydrolysed to their constituent amino acids. Deamination of three of the most common of such amino acids—glutamic acid, aspartic acid and alanine—yield three keto-analogues which are constituents of the metabolic pathways described above, namely α-ketoglutaric acid and oxaloacetic acid (Krebs cycle substrates), and pyruvic acid (glycolytic end-product), thus providing three points of entry to the system. Many other amino acids can be converted to one or other of the three, and in this way the bulk of protein foodstuff can be broken down to yield energy in phosphate linkage. The main difficulty is that the nitrogen which these substances contain has no place in the pathways described, which means that ammonia arises as a toxic end-product as indicated in Fig. 1.1.

There is a close relation between the different parts of the metabolic system and cellular architecture. Thus the process of oxidative phosphorylation is associated with the mitochondrion of the cell, and so is the oxidation of fatty acids. In Fig. 1.1, this association has been illustrated by enclosing relevant parts of the system within a dotted line representing the outer wall of the mitochondrion. The enzymes of the glycolytic pathway, on the other hand, are not associated with a structural framework, but occur free in solution in the cytoplasm, as do many of the transaminases involved in the interconversion and the deamination of amino acids, which precede their entry to the Krebs cycle.

Figure 1.1 illustrates that the continued production of high energy phosphate is dependent on the sustained input of one or more of the classes of foodstuff and of oxygen; and that the output from the system includes toxic products like ammonia and carbon dioxide, which must be detoxicated or removed, and heat, which must be dissipated. Water should strictly speaking figure as an input as well as an output, since many of the reactions concerned in the oxidation of foodstuffs involve a preliminary hydrolysis, that is, an addition of water to the molecule. However, the amount of water produced in the final stages of

oxidation greatly exceeds the input, so that in the overall reaction there is a net output, as represented in the figure. In view of the dependence of all metabolic systems on an aqueous medium as a basis for their function, this appearance of water as an end-product of metabolism may usually be regarded as an advantage. The quantity of "metabolic" water so produced differs according to the type of material oxidized, and the possible implications of such differences will be considered later in relation to the question of water balance.

With this general summary of metabolism as a basis, we may proceed to consider firstly the nature of insect metabolism, and secondly the various physiological processes like nutrition, respiration, excretion, osmoregulation etc., which serve to provide an appropriate input to the system, to deal with the output and in other ways to ensure the proper functioning of the metabolic machinery.

2. The Breakdown of Organic Molecules in Insects

Because of the small size of insects, and the correspondingly small quantity of material available for analysis, the study of insect biochemistry has not yet advanced to the level of comparable vertebrate studies. With the development during the present century, however, of a variety of microtechniques, notably those based on paper and thin-layer chromatography, it has become possible to isolate and assay extremely small amounts of different metabolites, and a picture of insect biochemistry is beginning to emerge as a result of these technical advances.

a. The Insect Mitochondrion

During recent years a great deal of work has been done on the insect mitochondrion, particularly with the flight muscle mitochondrion usually referred to as the sarcosome. These mitochondria constitute particularly favourable experimental material because of their large size, and their occurrence in closely packed rows between the intracellular myofibrils of the large muscle cells (see Fig. 1.2). Their isolation, in amounts adequate for experimental work, is easily and rapidly accomplished by grinding the thoraces of suitable insects with pestle and mortar, filtering off the coarse particulate material comprising cuticular and tracheal fragments mixed with partially dissolved myofibrils, through layers of muslin cloth, and spinning down the large sarcosomes at quite low centrifugal speeds. After washing of the sarcosome pellet and re-suspension in a suitable medium the properties of the mitochondrion can be studied; the rate of oxidation of various substrates can be determined on the basis of oxygen consumption, either by standard manometric methods, or with the more recently developed oxygen electrode; the oxidation and reduction of components of the hydrogen transport system can be studied by spectrophotometric
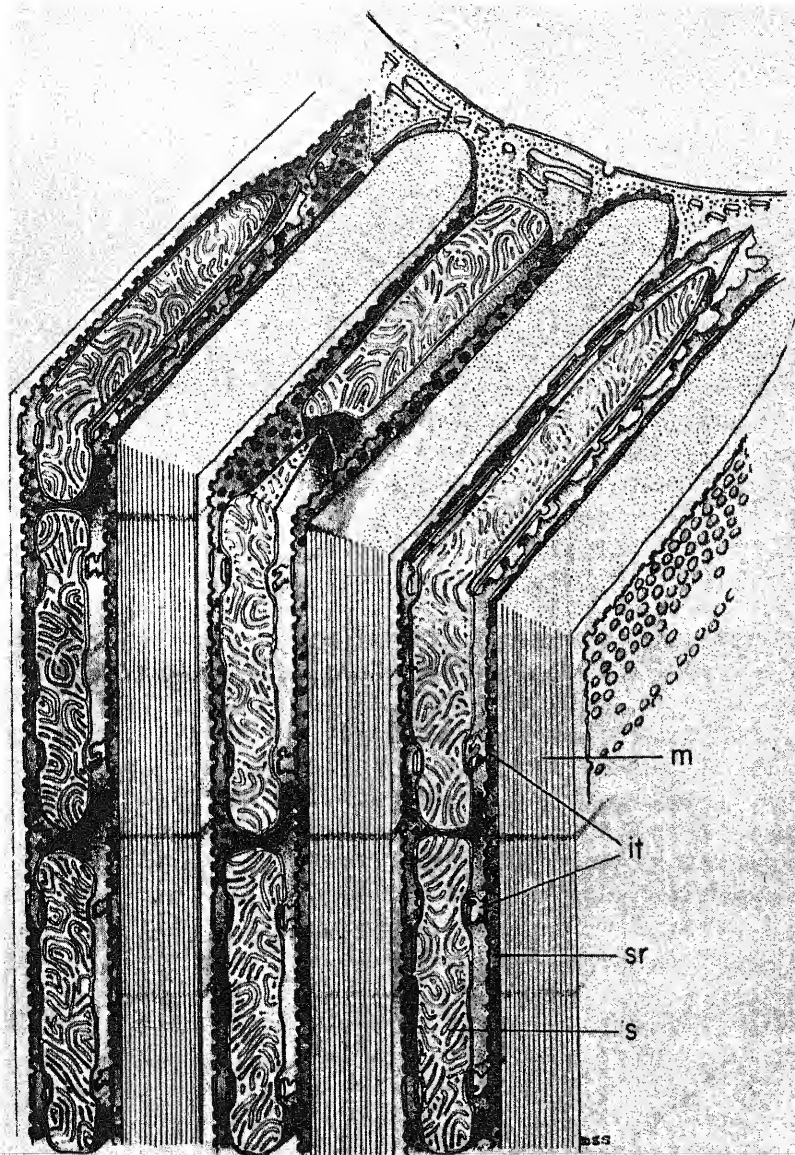
Fig. 1.2. Diagrammatic reconstruction of fine structure in the flight muscle of the dragon it, intermediary tubule; m, myofibril; s, sarcosome; sr, sarcoplasmic reticulum (Pringle, 5 after Smith).

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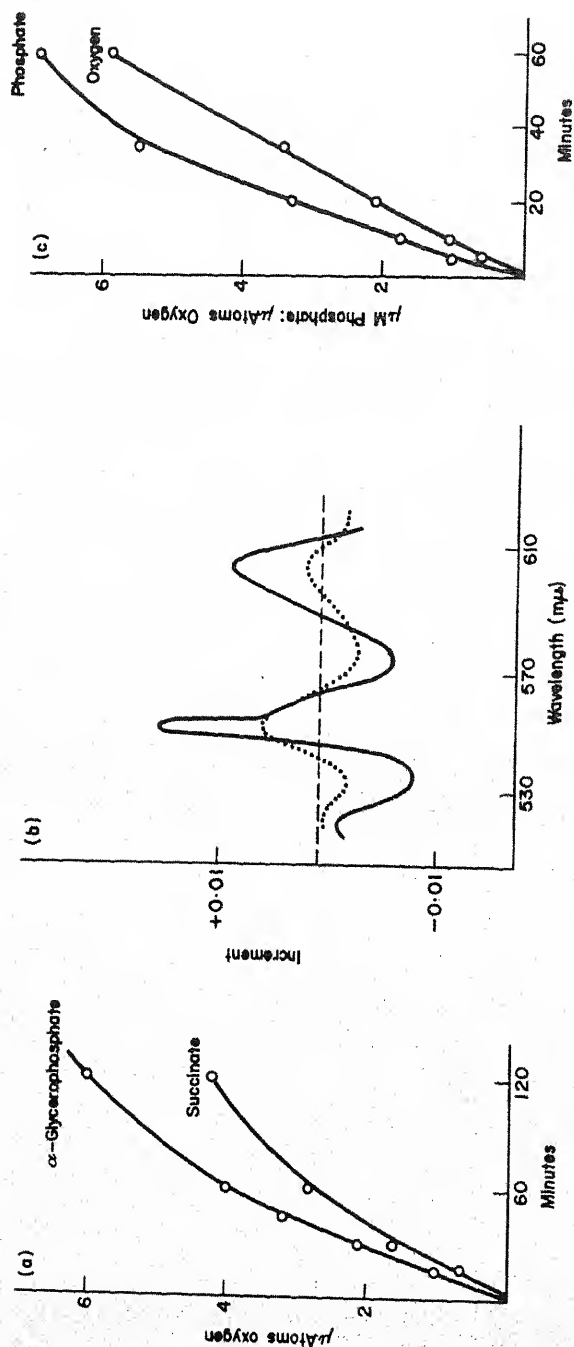
Fig. 1.3. Three different approaches to the study of mitochondrial activity. (a) The manometrically determined oxygen consumption of teased muscle preparation of the locust, showing the high rate of oxidation when α-glycerophosphate is provided as a substrate (data from Zebe et al., 1959). (b) The state of oxidation of mitochondrial constituents as shown by the split-beam spectrophotometer; the dashed curve represents the increment of absorption over a range of wavelengths between aerobic sarcosomes of the housefly in the absence of substrate (hydrogen carriers fully oxidized) and in the presence of α-glycerophosphate. The solid curve shows the difference spectrum in the absence of oxygen (hydrogen carriers fully reduced). The absorption peak of reduced cytochrome c (at 550 mμ) and of cytochrome a (at 605 mμ) are clearly seen under the steady state conditions of oxidation, showing that these pigments play a part in hydrogen transport during oxidation of α-glycerophosphate (Chance and Sacktor, 1958). (c) The esterification of phosphate and the uptake of oxygen by sarcosomes of the blowfly during oxidation of α-ketoglutarate. The P/O ratio (see text) averages about 1.5 for the experiment (Lewis and Slater, 1954).

means; and the ability to generate high energy phosphate bonds can be followed by determining the anhydride formation of inorganic phosphate with ADP to produce ATP (see Fig. 1.3).

In this context it should be noted that the mitochondrion is an extremely complex and delicate organelle, and one which is very liable to degenerative changes. During isolation careful precautions must be taken to guard against deleterious influences such as osmotic shock, excess calcium, accumulation of inhibitory factors etc. In fact, the properties of the mitochondrion depend to a rather uncomfortable extent on the method used for its isolation; even with all recommended precautions taken, one may hesitate to equate *in vitro* with *in vivo* performance, and experimental results have usually to be accepted with reservation.

All the enzymes of the Krebs cycle have by now been shown to occur in the mitochondria of different insects, and the ability of the insect mitochondrion to effect the complete oxidation of pyruvate has been amply confirmed. A number of other substrates have been tried, and α-glycerophosphate has been found to be a particularly good one, capable of being oxidized faster than pyruvate, and than members of the Krebs cycle itself, such as succinate (see Fig. 1.3(a)). This difference will be discussed further when the glycolytic pathway comes up for consideration.

The electron transport system has also been extensively studied particularly since the development of the split-beam spectrophotometer, which enables measurements to be made of the state of oxidation of hydrogen carriers in the intact mitochondrion (see Fig. 1.3(b)). The carrier system appears to conform to the basic pattern, as illustrated in Fig. 1.4. Hydrogen is passed on from substrate to flavoprotein, and from there through a series of cytochromes to oxygen. Three different flavoproteins have been shown to be involved in the transfer of electrons from different substrates to cytochrome b, their relative activities varying from tissue to tissue. In sarcosomes the α-glycerophosphate pathway appears to be particularly active, in accord with the results reported above, and another carrier, coenzyme Q appears to be interposed between the flavoprotein and cytochrome b.

It is generally agreed that during the transfer of electrons from NADH to oxygen by a carrier system of this sort, the reduction of one atom of oxygen is accompanied by the generation of three molecules of ATP from ADP and inorganic phosphate. Where the hydrogen is handed on directly from substrate to flavoprotein, as for succinate or α-glycerophosphate, the first phosphorylation, which occurs at the level of NADH oxidation, is lost, giving a theoretical maximum of two molecules of ATP per atom of oxygen. These phosphate/oxygen ratios, usually denoted as P/O, have been determined for a range of insect mitochondria, and it has proved possible during recent years to attain to values which approximate quite closely to the theoretical maximum (see Fig.
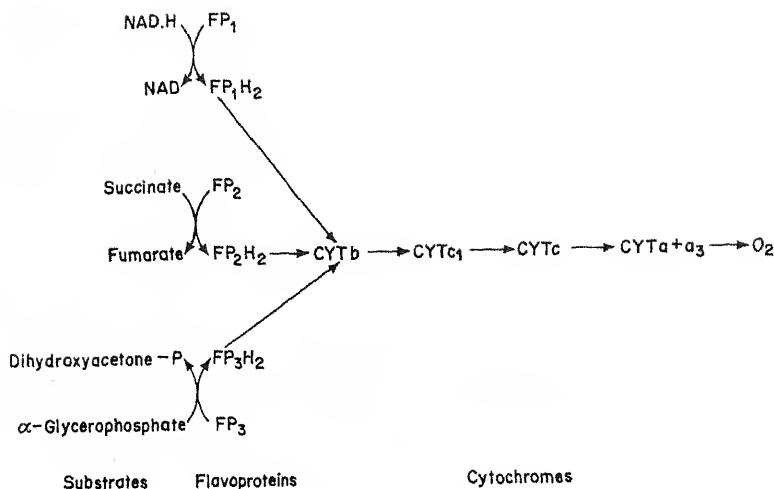
Fig. 1.4. Pathways of hydrogen and electron transport in the insect mitochondrion (from Gilmour, 1961).

1.3(c)), although results are rather variable. This may be a reflection of the difficulty of maintaining full functional integrity of mitochondria during the period required for isolation.

The problem of respiratory control has been the subject of a number of investigations with insect material, being particularly sharply posed with the mitochondrion of flight musculature; but the mechanism which is involved in the transition from the resting to the active state, associated sometimes with an increase in metabolic rate of more than 100-fold, is still not fully understood. With mammalian preparations the concentration of ADP has been shown to be of major importance in the regulation of oxidative phosphorylation. When work is done, as during muscular activity, ATP is converted to ADP, and the resulting increase in ADP concentration stimulates mitochondrial activity, and hence the reconstitution of ATP. With insect sarcosomes, too, ADP appears to exercise a measure of control, as shown in Fig. 1.5; during recent years respiratory control ratios (the ratio of rates of oxidation in the presence and absence of ADP) in the region of 30 have been demonstrated, but this still falls somewhat short of the degree of control shown by the mitochondrion *in vivo* during the transition from rest to flight. The remaining discrepancy could be accounted for in various ways, but none of the explanations so far advanced have been convincingly supported by experimental evidence. One possibility would be that the availability of glycolytic substrate for entry into the Krebs cycle might constitute a limiting factor; another that the level of concentration of the Krebs cycle substrates themselves might constitute a limitation, and that their concentration might be
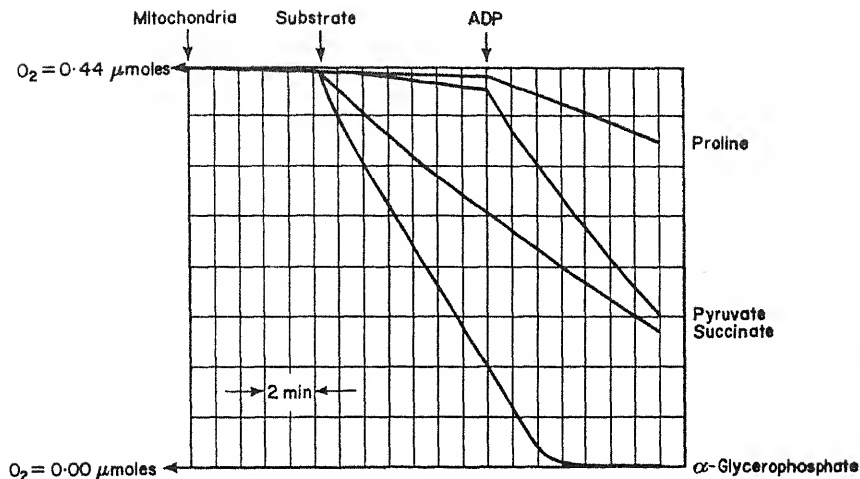
Fig. 1.5. Polarographic oxygen electrode records showing the decrease in oxygen concentration during incubation of blowfly mitochondria with different substrates. 0.1 mg of mitochondrial protein was added to 2.0 ml of a sucrose medium containing 1 mM Mg^{++}at the first arrow; oxygen uptake is negligible in the absence of substrate. At the second arrow the listed substrates were added at a concentration of 10 mM; α-glycerophosphate and succinate are oxidized at high rates, but oxygen consumption in the presence of pyruvate and proline remain low until ADP is added (at the third arrow); both show high respiratory control indices, while succinate and α-glycerophosphate oxidation is unaffected by addition of ADP (from unpublished records).

boosted during early phases of flight by deamination of appropriate amino acids whose keto-analogues could enter the cycle, thus making more oxaloacetate available for condensation with acetyl CoA, and hence allowing a higher rate of input to the Krebs cycle. It is possible that a combination of regulative mechanisms, of which the three here outlined may be examples, co-operate to achieve the high level of respiratory control which characterizes the insect sarcosome; on the other hand, it may be that failure to achieve the requisite level of control with ADP may be attributable to a process of deterioration during sarcosome isolation.

It is only during recent years that information has begun to become available on the oxidation of fatty acids, which constitute one of the main food reserves of many species of insect. Early work had shown that acetate could be oxidized by isolated mitochondria at reasonable rates, but it had not been possible to demonstrate substantial oxidation of higher fatty acids. Subsequently, it was discovered that carnitine esters of long-chain fatty acids were rapidly oxidized by insect mitochondria, and that the addition of carnitine to mitochondrial suspensions greatly enhanced the rate of oxidation of higher fatty acids, pointing to the existence in the mitochondrion of a system capable of synthesizing carnitine esters. The anomalous situation, that insect mitochondria appeared

unable to oxidize one of the most important of their natural substrates, has thus been resolved, but further work needs to be done to establish the precise role of carnitine and to extend the observations to a range of insect species.

Before leaving the subject of the insect mitochondrion, it may be well to sound a note of caution concerning the results obtained with isolated preparations. One of the most striking features of the history of investigations in this field is the apparent change in the properties of the sarcosome which has gone hand in hand with refinements of technique, particularly in relation to extraction media and procedure. Early sarcosomal preparations showed very poor oxidative capacity, and there appeared to be little discrimination by the mitochondrion between different substrates. There followed a period during which an apparent distinction could be made between the high rates of oxidation of α-glycerophosphate on the one hand and most other substrates on the other. With later preparations it has been shown that substrates like pyruvate, proline, NADH and succinate may be oxidized at rates equal to or exceeding those of α-glycerophosphate. Similar trends can be seen in relation to other mitochondrial properties; early investigators were unable to demonstrate substantial control by ADP, but at a later stage respiratory control indices were obtained that were little inferior to those which had been found to characterize mammalian preparations while at the present day it is possible to isolate sarcosomes which show levels of control far in excess of the mammalian mitochondrion. It may well be, however, that with further refinements of technique, based on a better understanding of the phenomena under investigation, we may have yet again to revise our views on *in vivo* mitochondrial function.

b. Glycolysis

Glycolysis in insects, as in other animals, is mediated by enzymes associated with the soluble fraction of tissue homogenates. If the flight musculature of an insect, for instance, is homogenized in water and then subjected to high speed centrifugation, all particulate material can be spun down, not only gross fragments, nuclei and mitochondria, but also a finely particulate "microsomal" fraction, representing remains of the intracellular endoplasmic reticulum. The clear supernant then contains only soluble material, including certain enzymes and co-factors and most of the substrates. It is in this solution that enzymes mediating the breakdown of glycogen to 3-carbon fragments can be found. To demonstrate their presence, the soluble extract is usually dialysed in order to remove endogenous substrates. A particular substrate, for instance glucose-6-phosphate can then be added to the extract, and its transformation to the reaction product glucose-1-phosphate demonstrated, indicating the presence of the corresponding enzyme phosphoglucomutase. Since dialysis of the soluble fraction removes not only substrates but also other small molecules in solution,

such as NAD, ATP, inorganic phosphate etc., it is necessary to add appropriate co-factors and ions in order to demonstrate the occurrence of those reactions that require them.

In this way all enzymes of the glycolytic pathway have been shown to be active in extracts from a variety of tissues and from a number of different species of insect. These investigations have also demonstrated one of the most striking peculiarities of insect metabolism, the importance, namely, of a special carbohydrate, trehalose, which occurs as a side-branch of the normal metabolic pathway; this substance appears to play a far more important part in the metabolism of insects than it does in other animals which have been investigated.
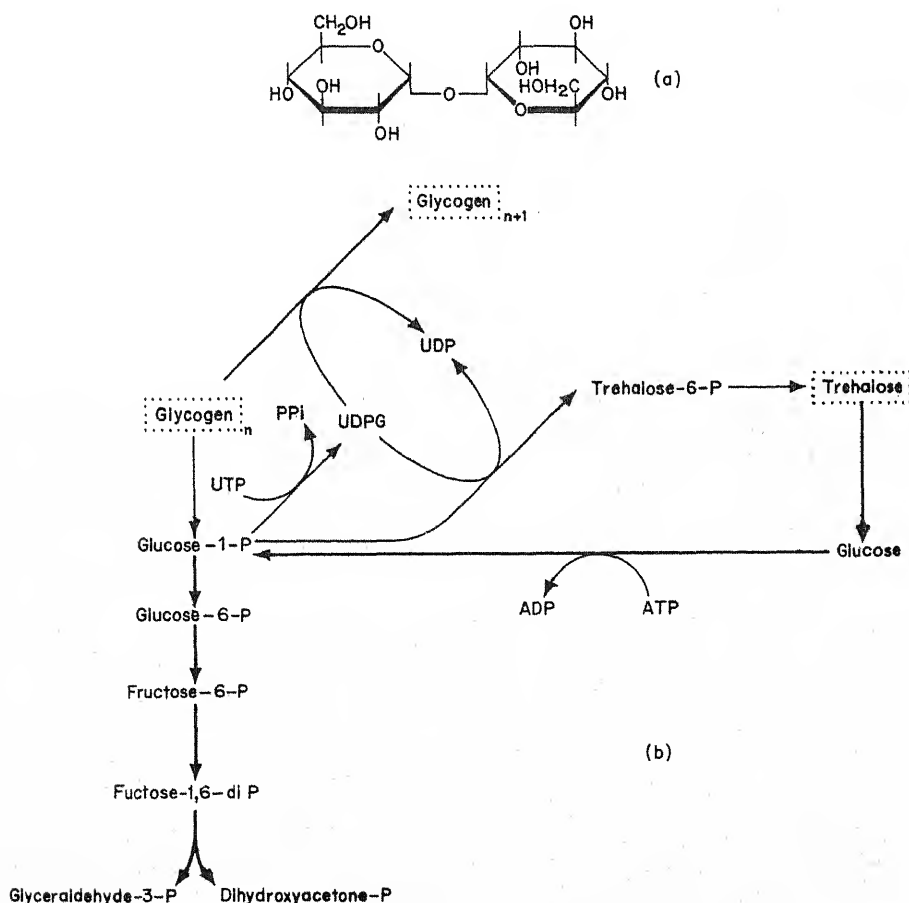
Fig. 1.6. The structure of trehalose (a) and its relation to the glycolytic pathway (b). UTP, uridine triphosphate; UDP, uridine diphosphate; UDPG, uridine diphosphoglucose; n, number of glycosyl units; PP_i, inorganic phosphate.

The structure of trehalose, and its relation to the general glycolytic pathway is illustrated in Fig. 1.6. Trehalose is a disaccharide composed of two glucose units joined through a 1,1 α-linkage. It can be hydrolysed to its constituent glucose by the enzyme trehalase, which has been shown to be active in many insect tissues, particularly high activities being recorded in the alimentary canal. Trehalase is also capable of catalysing the synthesis of trehalose, but this pathway is probably not of physiological significance. As shown in the figure, the normal precursor for synthesis is glucose phosphate and uridine diphospho-glucose (UDPG), which is a complex of glucose with uridine diphosphate, a nucleotide analogous to ADP, but with uridine instead of adenine as the organic base. This complex is a normal intermediary in the synthesis of glycogen, constituting the mechanism by which glucosyl residues are added to pre-existing glycogen chains. This nucleotide complex may, under the influence of the enzyme trehalose-6-P-synthetase, effect the coupling of a glucosyl unit to glucose-6-phosphate, instead of to glycogen, to produce trehalose-6-phosphate, and this, in turn, can be dephosphorylated by trehalose-6-phosphatase to yield trehalose itself.

Trehalose makes up a considerable proportion of the carbohydrate reserve in insects, and occurs in particularly high concentration in the blood, or haemolymph, of resting insects, where values between 0.5 and 5.0 g/100 ml of blood are commonly encountered (see Chapter 3). During flight there is a sharp fall in trehalose concentration, and this, together with a great deal of other evidence, suggests that trehalose constitutes a readily available substrate for metabolism generally and for flight metabolism in particular, as indicated by heavy arrows in Fig. 1.6. The occurrence of this subsidiary reserve in the haemolymph stands in marked contrast to the situation in vertebrates, where blood sugar is maintained at a low level of concentration (usually less than 0.1 g/100 ml). Such a substantial haemolymph reserve would be of particular significance in relation to the heavy demand for substrate which would characterize active flight musculature, especially in view of the relatively inefficient open type of circulatory system in insects (Chapter 3), for it would ensure that a steep concentration gradient would be available to promote the rapid diffusion of substrate across the sarcolemma to reach the oxidative machinery. A simple requirement for rapid diffusion would, however, be better met by a high concentration of glucose, since the glucose molecule is smaller than that of trehalose, and therefore capable of faster diffusion. Considerations of this kind could not, therefore, account for the introduction of trehalose as a respiratory substrate. It is possible that the occurrence of trehalose as the main blood sugar is related not so much to the utilization of carbohydrate as to its uptake from the gut. The presence of high concentrations of glucose would militate against the absorption of this food material, which is a major element of the diet in many insects. Further discussion of this possibility will be deferred till the problem of digestion is considered (Chapter 2).

The occurrence of trehalose as a major element of carbohydrate metabolism is not the only peculiarity of the glycolytic system in insects. What may be considered as a second side-branch of the main reaction sequence is particularly well developed in this group, namely the reduction of dihydroxyacetone phosphate to α-glycerophosphate under the influence of the enzyme α-glycerophosphate dehydrogenase. This enzyme is particularly active in flight musculature, where its occurrence appears to be linked with an apparent defect of the mitochondrial system to which reference has already been made; the inability, that is, of sarcosomes to effect the rapid oxidation of NADH.

One of the steps in the glycolytic transformation of triosephosphate to pyruvate involves a dehydrogenation with NAD acting as hydrogen acceptor. Since NAD is present only in catalytic amounts, the reduced form must be oxidized as rapidly as it is formed if the process of glycolysis is to continue. With the high activity of α-glycerophosphate dehydrogenase in sarcoplasm this re-oxidation can readily be achieved, but in itself this would confer little advantage since, as can be seen from Fig. 1.7, the net yield from the reaction would be zero; the two molecules of ATP which are required for the early phosphorylations are just balanced by the yield of ATP involved in the transformation of triosephosphate to pyruvate, and the NADH formed at one point of the reaction sequence would have to be oxidized at the expense of a reduction of dihydroxyacetone phosphate to α-glycerophosphate. It is only
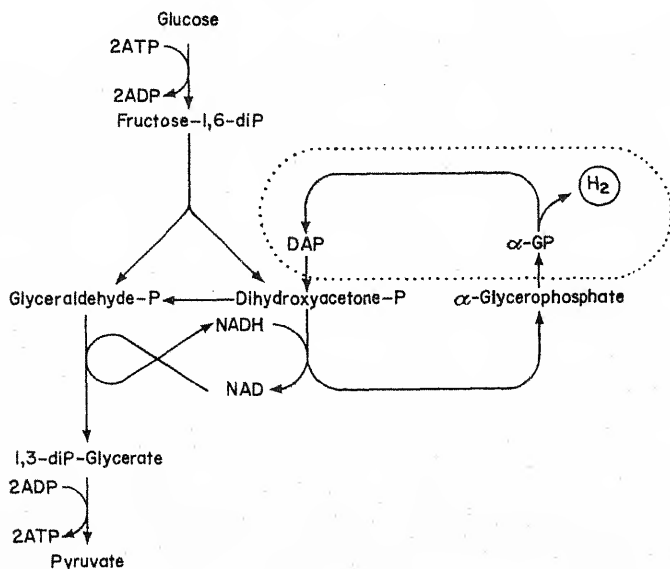
Fig. 1.7. The α-glycerophosphate shuttle system. DAP, dihydroxyacetone phosphate; α-GP, α-glycerophosphate. The dotted line represents the mitochondrion (for further explanation see text).
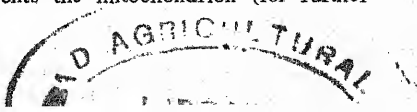
when this scheme is seen in relation to the capacity of sarcosomes for rapid oxidation of α-glycerophosphate that its significance becomes clear. The α-glycerophosphate formed in the sarcoplasm penetrates into the mitochondrion where it is oxidized to dihydroxyacetone phosphate under the influence of the mitochondrial enzyme (see Fig. 1.3(a) above). The dihydroxyacetone phosphate diffuses back into the sarcoplasm, where it becomes available for the oxidation of another molecule of NADH. What is effectively happening is that α-glycerophosphate serves to carry hydrogen into the mitochondrion, where it can be harnessed to the process of oxidative phosphorylation. Since this transport is in the nature of a shuttle service, it can operate on the basis of relatively small quantities of carrier, so that the major proportion of triosephosphate can be directed to the formation of pyruvate, and the over all reaction sequence becomes

$$glucose \rightarrow 2 \text{ pyruvate} + 2ATP$$
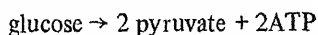
with normal glycolytic yields of energy, instead of as before

$$glucose \rightarrow \text{pyruvate} + \alpha\text{-glycerophosphate}$$
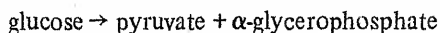
with zero yield of energy.

It may be permissible to speculate that, in the operation of this system, we see another adaptation to the requirement for a rapid release of energy necessitated by the activity of flight. The rate at which reduced coenzyme can penetrate the mitochondrion and become oxidized, while adequate for normal energy requirements, might not be capable of supporting the 100-fold increase in metabolic rate which characterizes flight; under these circumstances there would be need for a special mechanism to ensure a rapid transfer of hydrogen to the oxidative machinery; the simpler alternative, which would be to increase the permeability of the mitochondrion to NAD, might be impracticable in view of the necessity to maintain high concentrations of NAD inside the mitochondrion for intramitochondrial hydrogen transport.

c. The Oxidation of Amino Acids

The protein amino acids occupy a special place in degradation metabolism in so far as they contain between 10% and 34% of nitrogen. This nitrogen must be removed before they can enter the paths of oxidative metabolism and their energy trapped in usable form. The utilization of amino acids as a source of energy in insects is of particular interest in view of the high concentration of amino acids which characterizes insect haemolymph. There is considerable variation between and within species, but it is generally of the order of 1.5 g/100 ml as compared with values for vertebrate blood of about 0.03 g/100 ml (see Chapter 3).

A considerable amount of careful work has been done on quantitative aspects

of amino acid composition in insect haemolymph, but the results do not lend themselves to any sort of convincing generalization. With few exceptions, any one of the normal protein amino acids may occur in quite high concentration in one insect or another, and details of the amino acid pattern may vary widely from species to species and between different stages in the life history of a single species. What may be of significance in relation to an aspect of respiratory metabolism to which reference has already been made is that proline (strictly speaking an imino rather than an amino acid) usually figures as a dominant component, often with glutamic acid as its nearest rival, and between them reaching levels of 1.5 g/100 ml (cf. trehalose at 0.5-5.0 g/100 ml). Since an enzyme system which catalyses the oxidation of proline to glutamic acid has been shown to be active in insect mitochondria, and since the deamination of glutamic acid to α-ketoglutaric acid, a member of the Krebs cycle, is readily accomplished by many insect preparations both soluble and particulate, it may be that these two substances, proline and glutamic acid, should be regarded as Krebs cycle "primers", capable of being drawn into the Krebs cycle at the onset of flight and so augment the capacity of this enzyme/substrate complex for pyruvate oxidation. In the insects whose flight metabolism has been investigated it has been shown that the concentration of haemolymph proline declines sharply during early phases of flight, in accord with such an interpretation.

The main prerequisite of amino acid utilization in general is the removal of nitrogen, which can be accomplished by a process of oxidative deamination under the influence of certain flavoprotein oxidases, specific for each of the amino acids. However, the available evidence suggests that this may not be an important pathway in insects. Amino acids are optically active substances which may occur in two isomeric forms, the dextro- and the laevo-rotatory (D- and L-forms), and to these there corresponds the appropriate D- and L-amino oxidases. Naturally occurring amino acids are almost exclusively of the L-form, yet L-amino acids are deaminated slowly, if at all, by insect tissue extracts, while D-amino oxidases are relatively active. Whatever the significance of the occurrence of D-amino oxidation, it is clear that oxidative deamination of endogenous amino acids does not contribute materially to their metabolism. The only exception is glutamic acid which is oxidatively deaminated by a highly active glutamic dehydrogenase, present in both soluble and particulate fractions, and with NAD as the hydrogen acceptor

$$\text{NAD} \quad \overset{\text{glutamate} + H_2O}{\underset{\alpha\text{-ketoglutarate} + NH_3}{\times}} \quad \text{NADH}$$
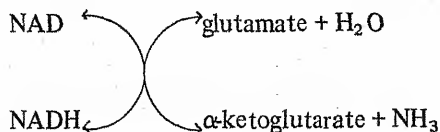
This system occupies a central position in the amino acid metabolism of insects, by virtue of the existence in most insect tissues of a variety of
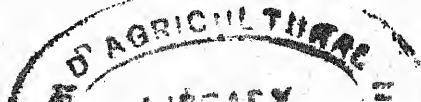
transaminases—or amino transferases—catalysing the transfer of amino groups
from amino- to keto-acids. When α-ketoglutaric acid acts as the amino-acceptor,
and the glutamic acid so formed is oxidatively deaminated, the over-all effect is
to provide for the oxidative deamination of the original donor molecule, e.g.

$$\text{alanine} + \alpha\text{-ketoglutarate} \xrightarrow{\text{transaminase}} \text{pyruvate} + \text{glutamate}$$

$$\underline{\text{glutamate} + \text{NAD} + \text{H}_2\text{O} \xrightarrow{\text{dehydrogenase}} \alpha\text{-ketoglutarate} + \text{NADH} + \text{NH}_3}$$

$$\text{alanine} + \text{NAD} + \text{H}_2\text{O} \longrightarrow \text{pyruvate} + \text{NADH} + \text{NH}_3$$
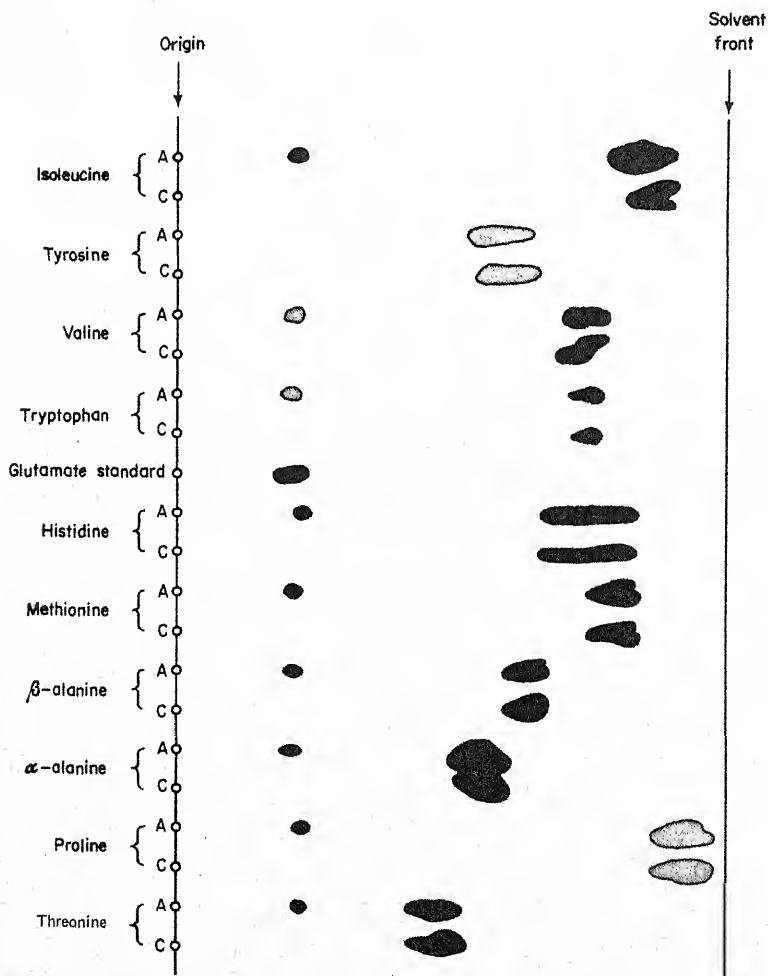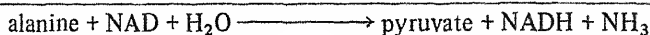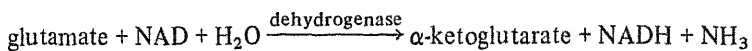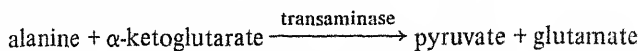
Fig. 1.8. Paper chromatogram showing the transamination of a number of amino acids
with α-ketoglutaric acid, leading to the formation of glutamate, by cockroach homogenates.
A, active test; C, control containing boiled homogenate (McAllan and Chefurka, 1961).
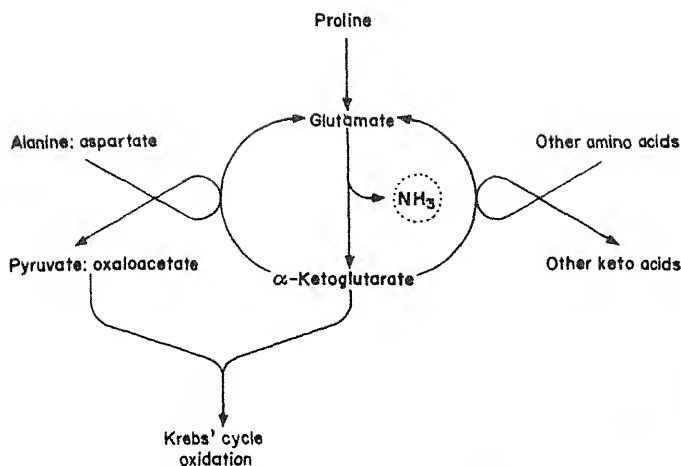
Fig. 1.9. Summary of amino acid degradation, to illustrate the central importance of glutamic dehydrogenase. For further explanation see text.

Transaminases active with α-ketoglutarate as an amino acceptor have been demonstrated in a number of different insects and operative with a number of different donor amino acids (see Fig. 1.8). The enzymes which catalyse the transfer of amino groups from aspartate and from alanine, whose keto-analogues, oxaloacetate and pyruvate, occupy positions close to the centre of degradation metabolism, are usually considerably more active than those involving other amino acids. But whatever the particular amino acid, it seems likely that this sort of coupling of transamination with oxidative deamination of glutamic acid, as summarized in Fig. 1.9, constitutes the main link between the amino acid pool and the energy releasing process of oxidative phosphorylation.

3. The Intermediary Metabolism of Insects

So far discussion has been limited to the breakdown of different kinds of organic compounds with capture of a proportion of the energy released during breakdown. A lot of metabolic activity, however, is directed not to the breakdown of compounds but to their synthesis—to the elaboration of special kinds of material disposed within the cell or secreted from it—and required for a variety of special purposes. Metabolic transformations of this kind may conveniently be grouped under the general heading of intermediary (or intermediate) metabolism. This involves an extensive area of enquiry and for present purposes consideration will be limited to a few selected aspects.

a. Storage Metabolism

The materials, whose breakdown serves for the supply of energy to insects, derive ultimately from ingested food materials which are absorbed from the gut

as soluble molecules. A proportion of these undoubtedly go to satisfy immediate metabolic requirements, but any surplus would be diverted to food depots for storage against a time of need. The main storage depots of insects are the so-called fat bodies, widely distributed in the body and dispersed in cellular sheets between its tissues (see Chapter 4). Since the storage of large quantities of soluble material would raise serious osmotic problems, it is not surprising to find that surplus foodstuffs are stored in cells of the fat body mainly in insoluble form, either as the polysaccharide glycogen or as neutral glyceride or fat.

The formation of glycogen has been shown to involve successive addition of glucosyl units to a preformed glycogen chain through the mediation of a nucleotide complex (see Fig. 1.6 above); and the mobilization of glucose from the polysaccharide involves phosphorolytic cleavage leading to the formation of glucose-1-phosphate which is subsequently converted to trehalose for transport to sites of respiration. It is unfortunate that, despite recent advances in the field of lipid chemistry, there is no comparable information about the synthesis of storage lipids or of the mechanism of their mobilization from storage depots. A certain amount is known of the nature of storage lipids: that they are neutral lipids whose fatty acid constituents comprise both saturated and unsaturated members with chain-lengths predominantly between 14 and 20 carbons. Lipases, capable of hydrolysing the ester linkage between fatty acid and glycerol, have been demonstrated in the fat body of insects, and this reaction presumably constitutes the first step in the mobilization of stored fats. But whether the higher fatty acids enter the haemolymph as such, or whether they are subjected to preliminary degradation first has not yet been determined. In general the biochemistry of lipids in insects stands in urgent need of thorough investigation with modern techniques.

b. Detoxication Metabolism

Carbon dioxide and ammonia have been shown to arise as principal end-products of metabolism (see Fig. 1.1). Both are toxic and require to be removed or detoxicated if malfunction of the metabolic machinery is to be avoided. The elimination of carbon dioxide takes place across the respiratory surface to which it is transported principally as bicarbonate. The initial reaction leading to bicarbonate involves the combination of carbon dioxide with water to form carbonic acid, and in many animals this is catalysed by the enzyme carbonic anhydrase. This enzyme appears to be absent or poorly developed in insects, so that the removal of carbon dioxide does not constitute a metabolic process, and it may best be discussed in connection with the general question of respiratory exchange.

It would appear that the toxicity of ammonia is such as to preclude disposal by simple diffusion in most terrestrial animals. The internal concentration required to ensure a sufficiently high rate of diffusion across relatively

impermeable surfaces or along extensive and tortuous diffusion paths of the tracheal system would probably exceed the limits of tolerance, and for this substance it has therefore been necessary to arrange for an alternative method of disposal. This has involved incorporation of the ammonia into a relatively non-toxic molecule, and in insects the principal vehicle for the removal of waste nitrogen is uric acid or substances closely related to it. Like other detoxication products, such as urea, these purines are non-volatile and hence cannot be eliminated by diffusion, and the function of elimination has been taken over by the excretory system (see Chapter 5).

The incorporation of amino acid nitrogen, or of nitrogen deriving from other sources, into the uric acid molecule has been extensively studied in vertebrates, and the synthetic pathway has been fully elucidated. The process is one of considerable complexity, involving a progressive build-up of the purine ring system from molecules or fragments of molecules deriving from quite complex precursors including glutamine, the amide of glutamate, and aspartate. Each step in the synthesis requires the use of ATP as a primer for the reaction, and the expenditure of high energy phosphate may be regarded as the price of detoxication. The derivation of different parts of the uric acid molecule was established by administration of radioactive isotopes of carbon and nitrogen in various forms (e.g. as glycine, formate, bicarbonate etc.) to the synthesizing system, followed by isolation and degradation of the uric acid produced, to establish the position of labelled atoms in the ring system. Figure 1.10 shows the structure of the uric acid molecule and the precursors of its different parts as established in this way.

Comparably exhaustive studies have unfortunately not yet been made of uric acid synthesis in insects. Early work based on the stimulation of uric acid synthesis by administration of possible precursors suggested pathways different from the ones which exist in vertebrates, but the evidence provided by such studies is equivocal. More recent work, based on injection of a radioactive precursor, formate-C^{14}, in the cockroach has shown incorporation of C^{14} in the 2- and in the 8-position, as expected on the basis of the vertebrate pathway. But it is too early to dismiss the possibility that the synthetic system of insects may differ from that of vertebrates in point of detail.

In many insects uric acid is virtually the sole end-product of detoxication (see Chapter 6), but in some there may be substantial degradation of the uric acid molecule by a series of uricolytic enzymes. The first step in this process is the production of allantoin, which involves opening of the imidazole ring under the influence of the enzyme uricase. A second enzyme, allantoinase, may produce cleavage of the pyrimidine ring of allantoin, leading to the formation of allantoic acid. In lower invertebrates degradation may proceed still further, to urea under the influence of allantoicase, and to ammonia under the influence of urease, but there is no evidence that either of these enzymes is active in insects.

It should be mentioned that uric acid may derive from another source than amino nitrogen. Purine nucleotides constitute important degradation products of nucleic acid, and following cleavage of the nucleotide to release the purine base, and deamination of the ring system, the purines can be oxidized to uric acid under the influence of an enzyme, xanthine dehydrogenase. The interrelationship between purine and alpha-amino pathways has been indicated in Fig. 1.10. What proportion of uric acid excreted derives from purine sources will depend to a large extent on the diet of the insect in question. It would be high in insects whose food consists largely of carbohydrates, low in insects whose diets include a lot of protein.
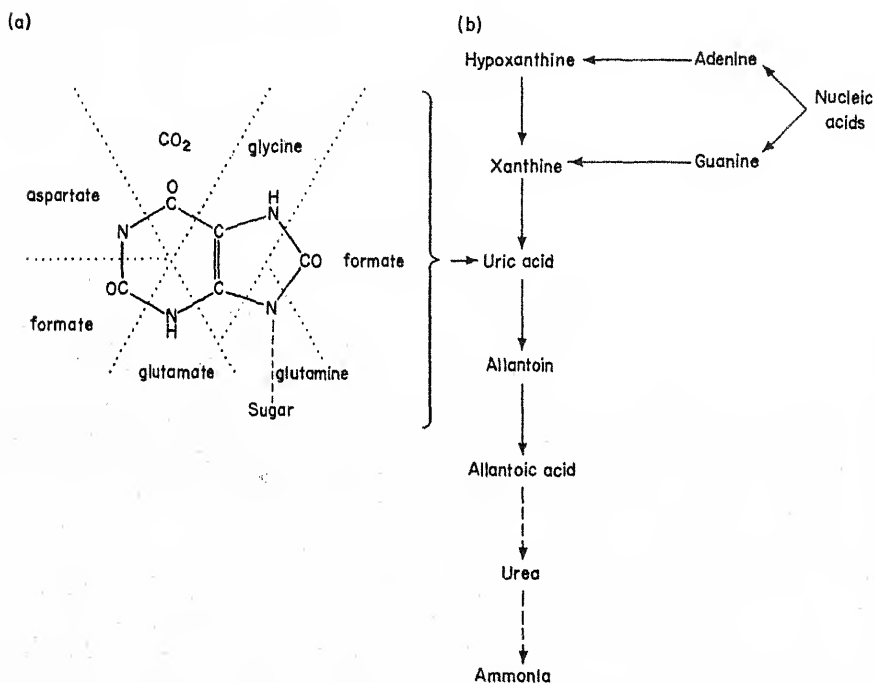
Fig. 1.10. Uric acid and its metabolic relationships. (a) The structure of uric acid in relation to its precursors. (b) Purine degradation.

Urea is a common detoxication product in certain, so-called ureotelic, vertebrates, and this substance can usually be recovered in small quantities from the excreta of insects. It is doubtful, however, whether its occurrence there should be taken as indicating a role in detoxication metabolism. The quantities involved are usually small in relation to the total output of excretory nitrogen, and there is evidence that the ornithine cycle, which is the pathway of its formation in ureotelic animals, is inoperative in the few insects which have been

investigated. Further work is necessary to establish the significance of excretory urea and the mode of its formation.

c. Cuticle Metabolism

The cuticle of insects functions both as an integument and as an articulated skeleton, and the material of which it is formed needs to embody a corresponding diversity of properties. To serve as a skeleton requires rigidity, yet at points of articulation the cuticle would need to be as flexible as possible. To serve as an integument in a terrestrial animal whose surface area is great in
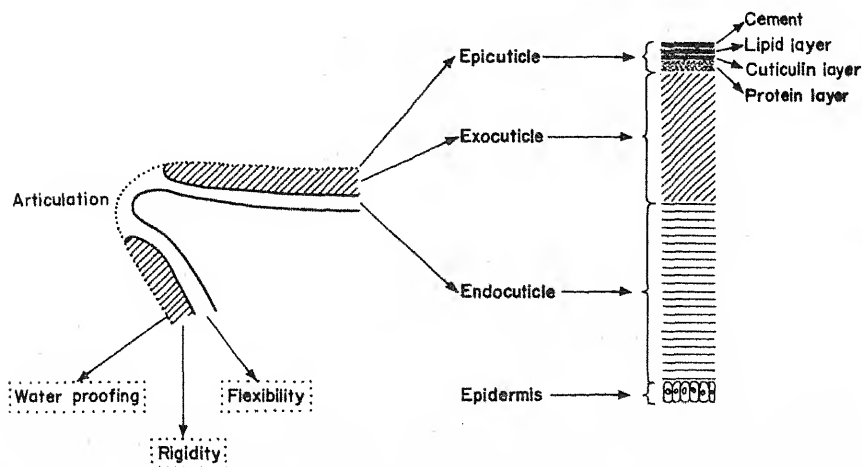
Fig. 1.11. Diagrammatic summary of the structure of the insect cuticle; the scale has been distorted to permit representation of the different layers of the epicuticle.

relation to its volume, water-proofing would be of paramount importance. These different requirements are taken care of by different layers of the composite cuticle, each with its own characteristics (see Fig. 1.11). Flexibility is a property of the relatively thick endocuticle which forms a continuous layer, as does the thin epicuticle that confers the property of impermeability to water; rigidity is a property of the exocuticle, which is discontinuous, and thus provides for points of flexible articulation between rigid regions. The properties of the different layers of the cuticle reflect their chemical composition, and this is summarized below. The way in which the cuticle is formed, through the activity of epidermal cells, will be described in Chapter 13 under the heading of Growth.

(i) The Endocuticle. The endocuticle is a lamellated structure composed principally of microfibrils of protein and of a characteristic polysaccharide, chitin. Recent work by Bouligand (1965) has indicated that the microfibrils are arranged in layered sheets, with the angle of fibril orientation in the tangential

plane changing between successive sheets in such a way as to produce the appearance of parabolically disposed fibres, as typically seen in sections of insect cuticle (see Fig. 1.12). Such an arrangement would allow for distortion in different planes, and thus contribute to the flexibility of the endocuticle. The protein fraction appears to comprise a number of different components, some freely soluble, some linked to the structural framework by relatively weak
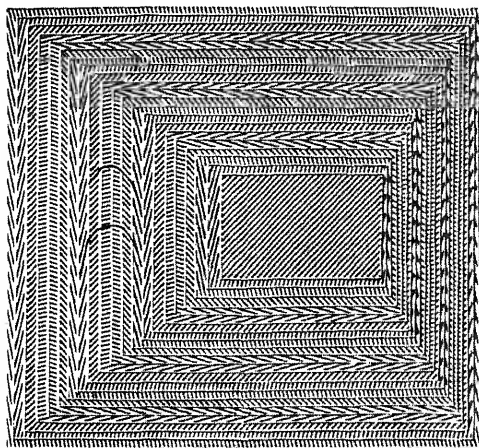
Fig. 1.12. A diagrammatic representation of the derivation of parabolic patterns in cuticle. A truncated pyramid of cuticle is viewed from the top. The chitin-protein microfibrils are arranged parallel to each other forming layers, and the direction of the microfibrils changes from layer to layer. Oblique sections (faces of the pyramid) then produce patterns with apparent parabolic microfibrils, two of which are indicated on the left face (Neville *et al.*, 1969 from Bouligand).

hydrogen bonds and van der Waal's forces, and some combined with chitin forming a glyco-protein complex. Chitin itself is a polysaccharide of high molecular weight, composed of unbranched chains of (1,4)-linked monomers of 2-acetamido-2-deoxy-D-glucose.
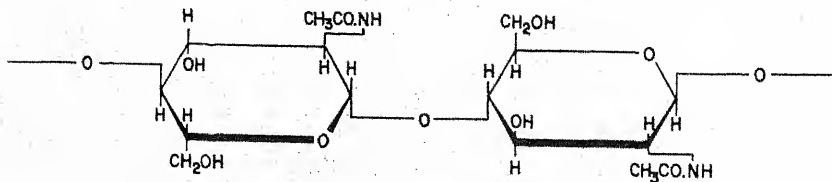
It is likely that a uridine diphosphate complex of N-acetylglucosamine is involved in its synthesis (cf. synthesis of glycogen, p. 20), but little is known of the details of the process in insects.

(ii) The Exocuticle. The exocuticle is a special region of the endocuticle

which has become stabilized and hardened by a process known as sclerotization. The chemistry of this process has been under intensive investigation for the past 20 years, and the general situation is clear although certain details are still imperfectly understood. It is conveniently studied during the formation of the puparium of flies, where extensive sclerotization takes place over a limited period of time, resulting in the transformation of the soft white maggot into the hard and black "pupa". At this time there is a substantial increase in the tyrosine content of the blood, and the activity of the enzyme phenolase, responsible for the oxidation of tyrosine and other phenols to their polyphenol analogues, also shows an increase. The dihydroxyphenylalanine (DOPA) formed by the oxidation of tyrosine (see Fig. 1.13) appears to be deaminated under the influence of enzymes in the blood, in the epidermis or in the cuticle itself, and the polyphenol so formed diffuses to the outer layers of the cuticle where it combines with oxygen in the presence of phenol oxidases, leading to the formation of the corresponding quinones. The quinones now diffuse inwards into the outer layers of the endocuticle, tanning the protein constituents, and converting the flexible chitin/protein complex into a rigid and hard material. The tanning itself appears to involve a reaction of the terminal amino groups of proteins to give N-catechol proteins which, in the presence of excess quinone, are oxidized to the corresponding quinonoid proteins. Further substitution can occur at other points of the benzene ring, and in this way bridges are formed between different protein molecules. These cross-connections are not necessarily confined to the ends of the protein molecules, since it has been shown that the terminal amino groups of lysine residues may become extensively cross-linked, giving stabilization of the whole lamellar complex, and preventing the sorts of deformations which can occur in the untanned cuticle.

Hardening of the cuticle by quinone tanning is usually, though not invariably, associated with darkening. The brown coloration is thought to arise when an excess of quinone is present; under these circumstances the quinones will tend to polymerize to form large pigmented molecules. Such polymers would have a large number of reactive sites, and would be capable of bridging large distances between protein chains.

The main element of controversy in the field of cuticle hardening concerns the precise nature of the tanning agent and the details of its combination with protein residues. A variety of closely related diphenols have been isolated from insect cuticle, but whether some or all of them are indeed involved in the process of tanning has not yet been unequivocally established; it has been said that nowhere in the field of cuticle chemistry has speculation been more popular, and further discussion would clearly fall outside the scope of the present work.

(iii) The Epicuticle. The epicuticle is the outermost layer of the cuticle, which may comprise no more than 5% of the total thickness. What it lacks in dimension, however, the epicuticle makes up in complexity. Four distinct layers
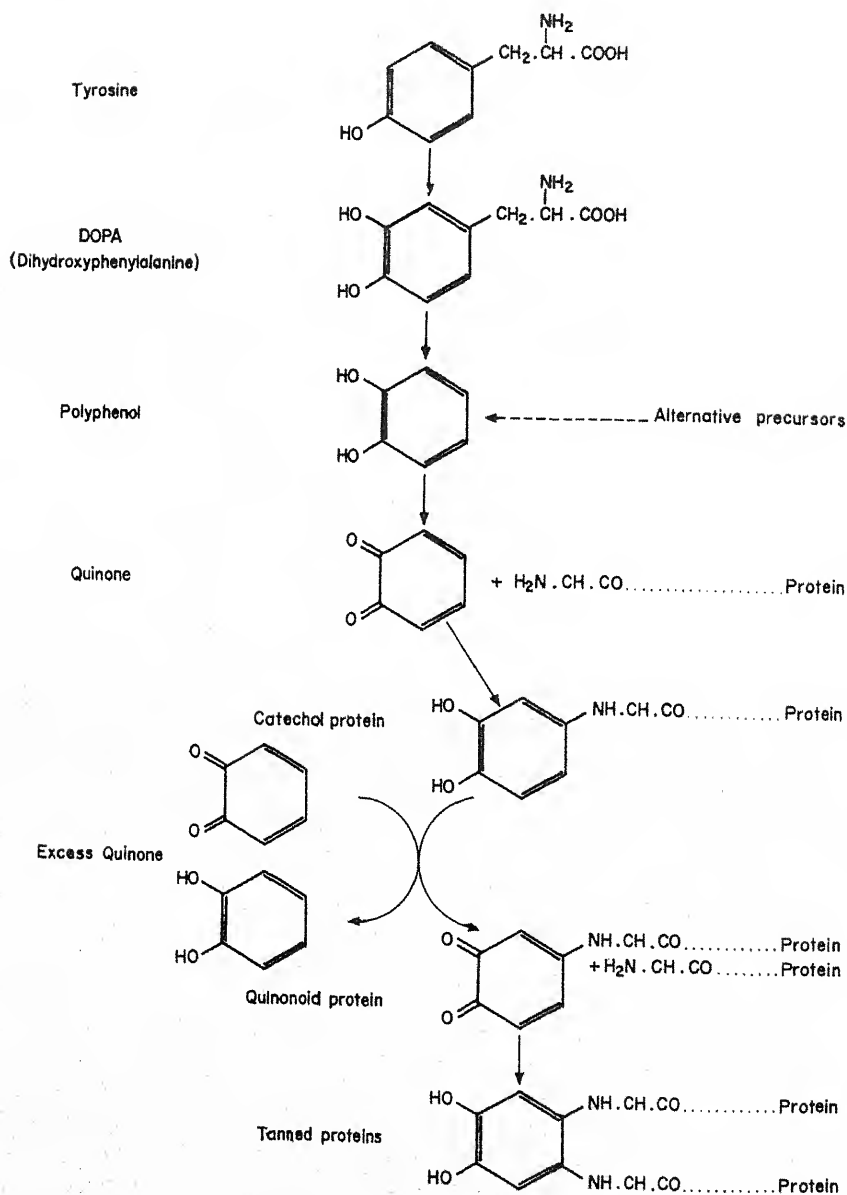
Fig. 1.13. Reactions involved in the hardening of insect cuticle.

can usually be recognized on the basis of suitable staining techniques (see Fig. 1.11):

(a) an outer layer of resin-like "cement";
(b) a layer of lipid;
(c) a cuticulin layer which forms a substrate for the lipid; and
(d) an inner layer composed apparently of tanned proteins impregnated with lipids, and containing no chitin.

Unfortunately the attenuated nature of the epicuticular layers has precluded detailed investigation of their chemical composition. The only component which has been subjected to rigorous analysis is the lipid fraction. In the insects so far examined the lipids have been found to comprise a complex mixture of different lipid classes, including usually a high proportion of saturated hydrocarbons with chain lengths ranging from C_{12} to C_{30}, and of free fatty acids and alcohols together with their esters. In addition a small proportion of aldehydes and of phospholipids have been recovered. There is good evidence that it is the lipid fraction which confers the property of impermeability to water, but it has not yet been established which of these various components are concerned in water-proofing, or whether different functions may be subserved by different constituents. The relation between cuticular lipids and water permeability will be discussed more fully in Chapter 16.

d. Pigment Metabolism

One of the most striking characteristics of the insects as a group is the diversity of pigmentation shown by some of its members. In many species colour has a physical rather than a chemical basis and depends on the structural conformation of surface elements which may produce interference colours or diffraction effects. But in many the colours result from the deposition of true pigments in the cuticle, or in the underlying hypodermis, and a variety of different kinds of pigment may be involved in the colour pattern of a given species.

(i) Melanins. The brown and black pigments of insects are usually assigned to the general class of "melanins", which as yet permits of no precise chemical characterization. One type of melanin forms when insect haemolymph is exposed to the air. In view of the presence of DOPA in the blood of insects, it seems likely that this black pigment constitutes a polymerized indole-derivative of this substance (see Fig. 1.14). In the cuticle, on the other hand, diphenols are probably deaminated before polymerization occurs, and here a pigment is formed which differs substantially from blood melanin in respect of the absorption spectrum. Finally, it appears that certain derivatives of tryptophan, such as kyneurenin and anthranilic acid, may undergo condensation reactions with quinones, and thus enter into the composition of pigment molecules. Thus a wide range of polymerization products is capable of forming, depending on the
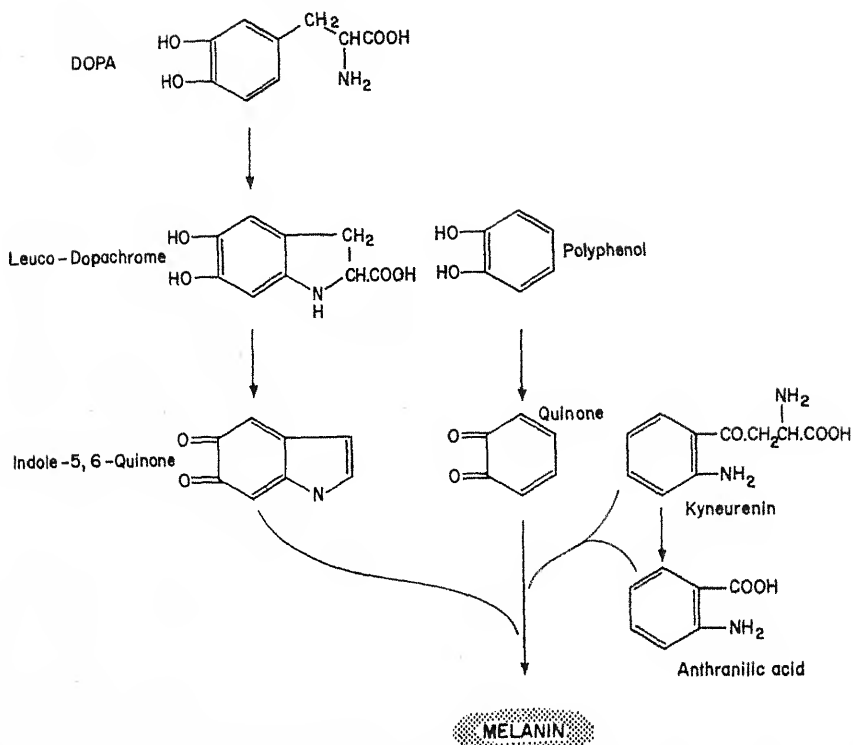
Fig. 1.14. The formation of melanic polymerization products from different precursors.

availability of different types of substrate, and the presence of the appropriate enzymes, the different types having in common their ability to absorb strongly a large range of visible light.

(ii) Pteridines. The pteridines constitute an important group of white, yellow and red pigments in the integument of different species of insect, and most strikingly associated with the epidermal scales of the wings of butterflies and moths. They usually exhibit strong fluorescence, which is a technical convenience, but many of them are photolabile, which has rendered structural investigations a matter of considerable difficulty.

The basic form of pteridine structure is given in Table 1.1, which also shows some of the different pigments produced by substitution at two positions of the ring. Experiments with radioactive tracers have suggested that the pigment may arise from purine precursors, by opening of the imidazole ring and subsequent combination with a dicarbonyl substance like glyoxylic acid.

In addition to their participation in general pigmentation, a number of these pigments have been isolated from the compound eyes of different insects. The

Table 1.1

The structure of pteridines

R^1	R^2	Pigment	Colour
OH	H	Xanthopterin	Yellow
OH	OH	Leucopterin	White
H	OH	Isoxanthopterin	Colourless
COOH	H	2-amino-hydroxypteridine-6-carboxylic acid	Yellow
OH	CH = C . CH$_2$OH | | OH OH	Erythropterin	Red

fact that some of them are extremely labile in the presence of light has raised the possibility that they may play a part in the physiology of vision, but details of such a process have yet to be elucidated.

(iii) Ommochromes. The ommochromes constitute another group of pigments which are involved in general pigmentation as well as being associated with the compound eyes of insects. Their function in vision appears to be confined to processes of adaptation and visual acuity, in so far as they occur in special pigment cells separate from the ommatidia (see Chapter 9). Their migration within these cells in response to changes in light intensity would provide a means of regulating the amount of light which reaches the photoreceptors.

The ommochromes are derivatives of the amino acid tryptophan, an example being the yellow pigment, xanthommatin, which has been isolated from the eyes of blowflies. The compound may be regarded as being a product of an oxidative condensation of two molecules of 3-hydroxykyneurenin, itself a derivative of kyneurenin, which in turn arises by oxidative decarboxylation of tryptophan (see Fig. 1.15).

Other ommochrome pigments, whose precise structure remains uncertain, are also formed from hydroxykyneurenin, as established on the basis of investigations with eye-colour mutants of various insects (see frontispiece). In certain species, for instance, it has been shown that a strain differing from the wild type by a single mutation has reddish-yellow eyes instead of the normal dark
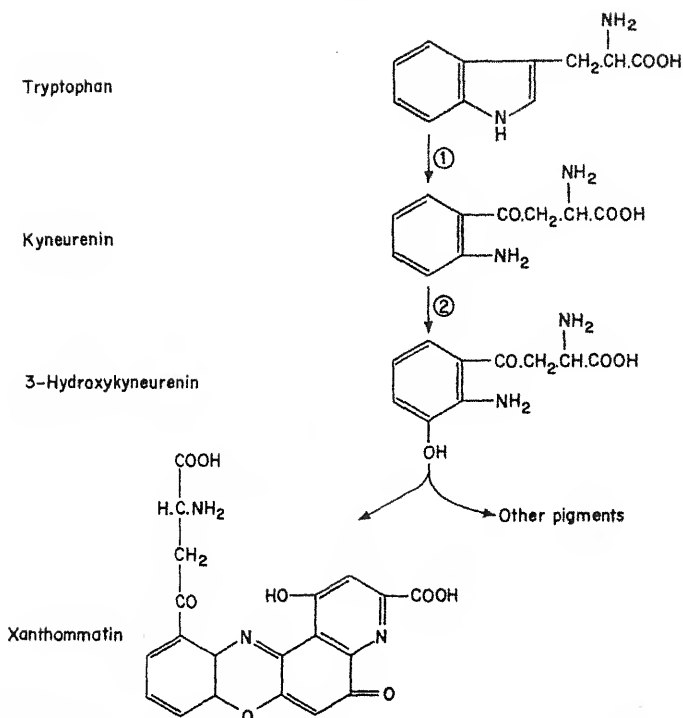
Fig. 1.15. Aspects of ommochrome metabolism. Reaction (1) is controlled by a single gene (v^+) and reaction (2) by another (cn^+).

coloration. Subsequently, it was established that in this mutant the enzyme responsible for the conversion of tryptophan to kyneurenin was inactive; the defect in pigmentation could be overcome by injection of kyneurenin into the developing insect. In another eye-colour mutant, normal pigment synthesis could be restored by the injection of hydroxykyneurenin, but not of kyneurenin, suggesting that here the enzyme responsible for the hydroxylation of kyneurenin was lacking. The indications provided by such studies concerning the pathway of ommochrome metabolism were subsequently verified by injection of radioactive precursors, and the demonstration of radioactivity in the pigments concerned. Thus the pattern of metabolism has been elucidated here by close co-operation between biochemists and geneticists.

THE SUPPLY OF FOOD

A metabolic system like that described in Chapter 1 requires an input of complex organic molecules to serve for the supply of energy, and to provide the raw materials necessary for repair and growth of the system itself; it requires, in other words, an input of food. This food is obtained from the environment, which contains an abundance of organic molecules of many different kinds, and there are few that do not provide sustenance for one or another species of insect. Most of the available food material occurs in the form of organic polymers, large molecules which cannot readily cross the cellular membranes, and are therefore not directly available to the metabolic system. They must be subjected to preliminary manipulation, involving mechanical and enzymatic breakdown, to get them into a form capable of being assimilated into the organism. This is achieved in the process of digestion, which is followed by the absorption of food materials into the body of the animal. But it is not enough that the metabolic system be kept supplied with complex organic molecules, it must be supplied with molecules of the right kind. For purposes of energy release any one of a variety of molecules will serve, but for purposes of maintenance and growth a number of quite specific requirements are involved. For it would seem that in the course of evolution insects, like other heterotrophic organisms which rely for their food on organic molecules elaborated by other organisms, have lost the ability to synthesize a number of essential components of their metabolic systems. In order to maintain their metabolic machinery, and in order to grow and reproduce, they must be supplied with a variety of specific molecules, which they need but have lost the power to make. The supply of food therefore involves a problem of nutrition, of the supply of the right kind of food, and this will be considered in the last section of this chapter.

1. Food Intake

The digestion and absorption of food takes place in the alimentary canal, which in insects is divided into three main parts (see Fig. 2.1(a)): the foregut (or stomodaeum) and the hindgut (or proctodaeum) are derived from ectoderm, and
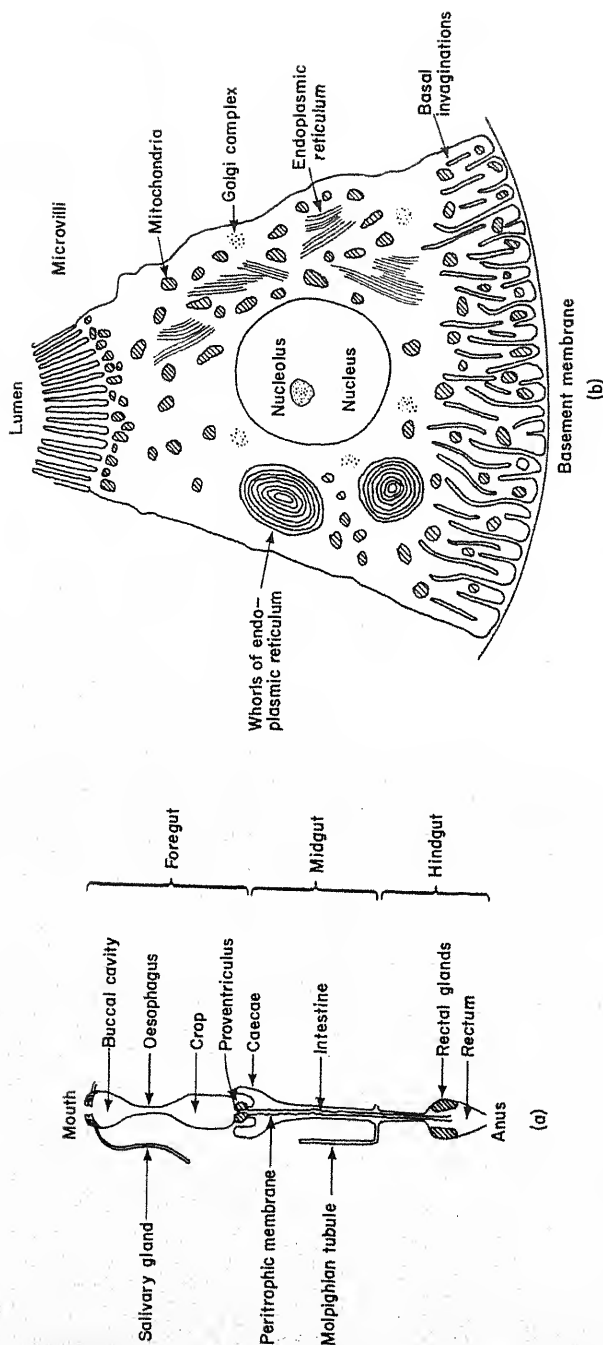
Fig. 2.1. (a) Diagram to illustrate the main features of the alimentary canal in insects. (b) Simplified diagram of a section through a cell of the midgut of the mosquito: on the left of the nucleus the endoplasmic reticulum is represented as it appears in the starved insect, on the right as it appears 24 hr after a blood meal (drawn from electron micrographs of Bertram and Bird, 1961).

Labels for diagram (b):
Lumen
Microvilli
Mitochondria
Golgi complex
Endoplasmic reticulum
Basal invaginations
Nucleolus
Nucleus
Whorls of endo-plasmic reticulum
Basement membrane
(b)

Labels for diagram (a):
Foregut
Midgut
Hindgut
Mouth
Buccal cavity
Oesophagus
Crop
Proventriculus
Caecae
Intestine
Rectal glands
Rectum
Salivary gland
Peritrophic membrane
Malpighian tubule
Anus
(a)

are provided with a lining of cuticle; the midgut (or mesenteron) is derived from endoderm. In many insects the midgut is lined by a so-called peritrophic membrane, but this lining is not produced by the cells of the midgut; it represents a secretion from a specialized region called the proventriculus, which marks the junction of foregut and midgut.

The division of the alimentary canal into major regions is usually reflected in a broad differentiation of function. The foregut, with associated structures such as mouthparts and other cuticular elements, is concerned to a large extent with the mechanical breakdown of food material, which may be a necessary preliminary to efficient enzymatic breakdown. It is often possible to distinguish a number of subdivisions of the foregut: a buccal cavity, for instance, which may be furnished with chitinous plates for triturition; a slender oesophagus; and a crop which may serve as a storage organ, and which may be more or less separated from the mainstream of food passage.

The midgut is largely concerned with the secretion of digestive enzymes, the breakdown of polymers under the influence of such enzymes and the absorption of digestive products and of water from the food mass. The ultrastructure of midgut cells is correspondingly complex (see Fig. 2.1(b)); each cell shows the typical folding of basal and peripheral membranes associated with absorptive function; and the endoplasmic reticulum exhibits pronounced cyclical changes, thought to be associated with the synthesis of digestive enzymes and their transport to the lumen. The midgut may carry a number of evaginations (gastric caecae) which serve to increase the surface area available for absorption.

The hindgut receives indigestible remains of the food and stores them pending evacuation. The situation in this region is complicated by the fact that excretory materials from the Malpighian tubules empty into the alimentary canal at the junction of midgut and hindgut, and further processing of the mixture of materials occurs in the rectum as a result of the activity of the rectal glands. Since this would appear to be associated primarily with processes of osmoregulation and excretion, rather than with those of digestion, it will be dealt with in another chapter (see Chapter 5).

The functional distinction which may be drawn between different regions of the alimentary canal is by no means absolute. Salivary glands, which may produce digestive enzymes, often open into the buccal cavity, so that a certain amount of digestion takes place in the foregut of many species in addition to the mechanical breakdown. Indeed in some insects saliva may be extruded on to the food mass before it enters the alimentary canal. On the other hand, a substantial amount of mechanical breakdown undoubtedly occurs as a result of the peristaltic contractions of the midgut, although the primary function of these contractions may be the onward propulsion of the food mass. Finally, it has been found that absorption of digestive products may occur in the hindgut as well as in the midgut of certain species of insect. Even so, the general

organization of the alimentary canal shows a surprising degree of uniformity in different species of insect. Variations on the common theme usually involve such things as the occurrence of a crop as a separate blind diverticulum of the foregut, and the presence or absence, and degree of development, of midgut caecae. It is only in the specialization of mouthparts that the sort of diversity normally associated with the insects as a whole is encountered. Arrangements range from the relatively simple grinding or shearing mouthparts of insects like the cockroach and the locust to the highly specialized adaptations shown by the blood-sucking mosquitoes or the nectar-feeding butterflies. A discussion of the details of such special adaptations would, however, lie outside the scope of the present work.

2. Digestive Enzymes

The bulk of food material available to insects is constituted by the three main classes of foodstuff—carbohydrate, protein and fat, in proportions depending on the diet of the different species. A corresponding diversity of digestive enzymes, capable of hydrolysing these different polymers to their constituent units, will therefore be required by most insects. Some of these enzymes are elaborated in special salivary glands and discharged into the buccal cavity, but most are produced by the cells of the midgut.

a. Carbohydrases

A great deal of work has been done to demonstrate the presence of different kinds of enzyme in the alimentary canal of insects, but investigations have seldom proceeded to the point of enzyme purification, nor have the conditions necessary for optimal activity been carefully established. Normally, crude extracts of digestive tract are tested for ability to hydrolyse specific substrates, maltose or trehalose for example, and if activity is detected, the presence of the corresponding enzyme, maltase or trehalase, is inferred. But what may be present is an enzyme capable of hydrolysing the α-glucosidic linkage generally, rather than maltose and trehalose specifically. Such results cannot, therefore, be accepted as evidence for the existence of two different enzymes; this could be established only by further work involving, perhaps, electrophoretic separation of protein components in the extract, and demonstration that hydrolysis of the two substrates is associated with different regions of the electrophoretic plate. In the absence of such data, and in the interests of simplicity, it will, for present purposes, be convenient to distinguish only a few general classes of carbohydrases.

(i) Glucosidases. Enzymes capable of hydrolysing the α-glucosidic linkage (as in maltose and trehalose) and the β-glucosidic linkage (as in cellobiose and gentiobiose) appear to be distributed widely among insects. α-Glucosidases are particularly active in herbivorous species, in accord with the fact that most of

the naturally occurring α-glucosides are of plant origin. The digestive enzyme which is active towards trehalose is probably different from the completely specific trehalase which has been discussed in the chapter on metabolism.

(ii) Galactosidases. Enzymes capable of hydrolysing the α-galactosidic linkage (as in melibiose and raffinose) have also been demonstrated in a range of insect species. β-Galactosidases, on the other hand, which would be required for the hydrolysis of carbohydrates like lactose, have been demonstrated in only a few species, of which the mealworm beetle is one.

(iii) Fructosidases. Sucrose is a β-fructoside, and enzymes capable of hydrolysing it have been isolated from the alimentary canal of a number of species of fly, but they do not appear to be widely distributed among insects generally.

(iv) Amylases. These enzymes split the storage polysaccharides of animals and plants (glycogen and starch respectively) to the disaccharide maltose, which is further degraded under the influence of α-glucosidases. Active preparations, differing considerably in respect of pH optimum which ranges from 5.5 to 9.5, have been isolated from the alimentary canal of a wide variety of insects.

(v) Cellulases. Cellulase activity has been demonstrated in a few species of wood-boring beetle, and in certain silverfish. Cellulose forms a substantial part of the diet in many insects, but this particular polysaccharide of glucose appears to be extremely resistant to hydrolysis, and most insects have to rely for its digestion on the presence of symbiotic microorganisms in the alimentary canal. Two other intractable polysaccharides, chitin and lichenose, are also capable of being digested by certain insects.

b. Proteinases

The digestion of protein in insects appears to follow the same general pattern as in other animals, with a preliminary breakdown of the large protein molecules, followed by an attack on the smaller peptones and polypeptides so formed, which are ultimately hydrolysed to their constituent amino acids; and in insects, as in other animals, a distinction can be made between different types of peptidase, some attacking from the carboxyl end, some from the amino end of the peptide, and some responsible for the hydrolysis of the dipeptide end-products of the previous attacks. Insect proteinases have been found generally to be more active in neutral or alkaline media than in acid, and such work as has been done on isolation and purification suggests that a number of different enzymes may be involved in the first stages of protein breakdown.

Of particular interest is the occurrence in certain insects of enzymes capable of attacking relatively resistant materials such as collagen and keratin. In keratin, the protein molecules are cross-linked by disulphide bridges associated with the cystine residues, and the ability to break the interconnections appears to be limited to a very few species of insect, belonging to quite different orders—the clothes moth, a dermestid beetle and certain biting lice of birds. The reduction

of the disulphide bonds effected by these insects appears to destroy the resistance of keratin to enzymatic attack and enables breakdown of the protein molecule by normal proteolytic enzymes.

c. Lipases

Enzymes capable of hydrolysing neutral fat to glycerol and free fatty acids have been demonstrated in a wide variety of insects, though not all the species examined have yielded active extracts. Most of the lipases concerned show maximum activity at alkaline pH values, and under these conditions the fatty acids released would form soaps, which would help to emulsify undigested fat, and thus facilitate further action of the hydrolytic enzymes.

The diet of the wax moth, *Galleria mellonella,* consists predominantly of beeswax, and the mechanism of digestion of this intractable material has been the subject of a number of investigations, but the situation has not yet been satisfactorily elucidated. It is thought that symbiotic microorganisms may co-operate in the process, which appears to be associated with an active phosphorous metabolism, but the significance of this association remains obscure.

This brief review of the digestive enzymes of insects has served to indicate that members of the class have the enzyme complement to deal with all of the three main classes of foodstuff, and that a number of species have developed special adaptations to special feeding habits which enable them to digest animal products, such as chitin, keratin, collagen and beeswax, and plant products such as cellulose and lichenose, which would normally be considered highly resistant to digestive attack. Over and above these special adaptations, it is often possible to see some correlation between general enzyme endowment and feeding habit. For instance, in carnivorous insects, such as certain species of blowfly, proteinases and lipases are strongly developed, while carbohydrase activity is feeble. Conversely, in plant-feeding beetles like *Melolontha* carbohydrases predominate over proteinases. But the diet of such insects, however narrow their food preference, is not completely restricted to a single class of food material, and the distinctions which can be drawn are only quantitative. Even in obligatory blood-suckers, like the tsetse fly, whose food contains negligible proportions of polysaccharide, digestive juices show weak amylase activity.

3. The Control of Digestion

Studies of digestion have produced evidence that the production of digestive enzymes may be under control of the neuroendocrine system in many insects, particularly in those whose feeding is markedly discontinuous. The release of hormones which stimulate the production of enzymes may be to some extent associated with the normal process of development, as in *Tenebrio,* the meal
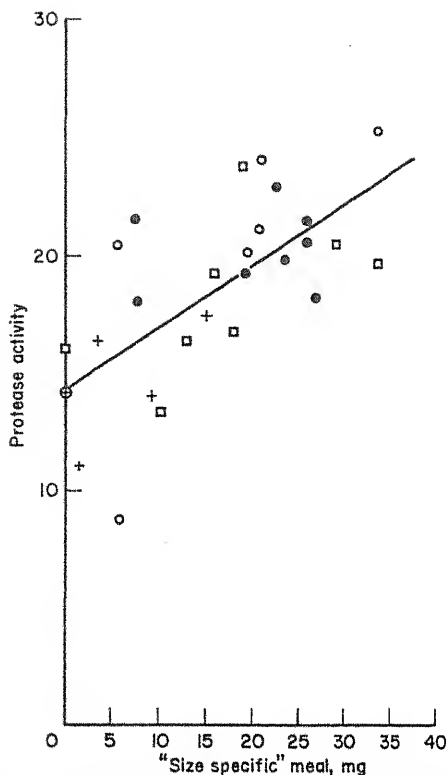
Fig. 2.2. The relationship between midgut protease activity and the size of the blood meal in the tsetse fly. ●, whole blood; ◻, 50% blood; ○, 25% blood; +, 10% blood (Langley, 1966).

worm beetle, where proteinase production is triggered by neuroendocrine release at emergence from the pupa. But in addition to such general control, there may be a process of regulation in relation to the act of feeding and to the amount of food ingested at feeding. In blood-sucking insects like black-flies and tsetse flies, feeding stimulates synthesis of proteolytic enzymes by cells of the midgut. In the tsetse fly the amount of enzyme produced, and hence the rate at which digestion takes place, is closely related to the size of the blood meal (see Fig. 2.2). The effect can be demonstrated whether the fly is fed on whole blood or on various dilutions of whole blood, suggesting that it is the volume of the meal, rather than the amount of protein it contains which is the important factor. It appears that the correlation between meal size and proteinase activity is mediated by stretch receptors situated in the wall of the crop and perhaps elsewhere, which monitor the degree of distension of parts of the alimentary canal.

4. Absorption

The absorption of digestive products from the lumen of the midgut has not been extensively studied in insects; discussion of the problem will therefore be based largely on a detailed series of investigations of the process as it occurs in the locust and the cockroach. In these, as in several other insects, the rate of absorption of food materials is controlled by the rate at which food is released from the crop, which in turn depends on the concentration of food in the contents of the crop, the volume of food material leaving it decreasing with increasing concentration. In order to study the process of absorption, it was necessary to eliminate this element of control, and this was done by injecting the food material into the alimentary canal by way of the hindgut. Changes in the concentration of material caused by absorption of water were established by incorporation in the food solution of the dye amaranth, a substance which is not absorbed by the insects under investigation. The use of radioactively labelled carbohydrate, amino acid and fat enabled ready determination of their concentration in the gut contents and in the haemolymph.

Fig. 2.3. Aspects of digestion in insects. (a) The percentage of material absorbed from different parts of the alimentary canal of the locust 15 min after the injection of glucose (20 mM) and of serine (35 mM). (b) Radioactivity on paper chromatograms of samples of the caeca contents and of haemolymph 15 min after injection of C^{14}-labelled glucose (20 mM) into the alimentary canal. The position of trehalose and of glucose standards is shown at the top of the figure. Data from Treherne (1958) and (1959).

Selected results of these investigations are illustrated in Fig. 2.3(a), which shows that the rate of absorption, both of glucose and of the amino acid serine, is greatest in the midgut caecae, appreciable in the midgut itself, and that little absorption takes place in the foregut or in the hindgut.

The process of absorption was found to be associated with an increase in the concentration of food materials in the alimentary canal, brought about by a withdrawal of water from the injected solution. Radioactive material in the gut lumen exchanged freely with unlabelled material in the haemolymph, but a net transfer of food from the gut to haemolymph was imposed by the concentration gradient associated with the higher gut concentration.

The rate of transfer of glucose was very much higher than the rate of transfer of amino acids. This difference was found to be correlated with the transformation of glucose to trehalose during passage across the gut wall, as illustrated in Fig. 2.3(b). The effect of this coupling of two molecules to form trehalose on the haemolymph side of the gut wall would be to maintain a low concentration of glucose in the haemolymph, and hence to ensure that a steep concentration gradient is maintained between gut and haemolymph, favourable for the rapid absorption of glucose. The larger size of the trehalose molecule, and its relatively low molar concentration, would to some extent reduce the back-diffusion of trehalose into the gut, so that the over-all effect would be a "facilitated" uptake of glucose.

The haemolymph concentration of amino acids tends to be high, hence the concentration gradient available for the transfer of material from gut to haemolymph remains relatively shallow. A diagrammatic summary of the situation is shown in Fig. 2.4.

It is possible that the occurrence of trehalose as the principal blood sugar in insects should be seen in part as a reflection of this mechanism of facilitated diffusion. Glucose constitutes a major dietary constituent for the majority of insects, and its uptake from the gut would be greatly hindered by the occurrence of high concentrations of glucose in the haemolymph. On the other hand, a high concentration of blood sugar is a primary requisite for the rapid transfer of substrate to actively respiring tissues generally, and to flight muscle in particular (see Chapter 1). These conflicting requirements are resolved by the interpolation of a secondary blood sugar, which enables a favourable concentration gradient to be maintained both for the transfer of glucose from gut to haemolymph, and for the transfer of substrate from haemolymph to the site of respiration. It may be that an analogous situation exists in the tsetse fly and perhaps in other insects that subsist on high protein diets. Here the main haemolymph amino acid, which may serve as a respiratory substrate, is proline; this substance is poorly represented in the food intake compared with substances like glutamic acid, aspartic acid, alanine and glycine, all of which stand in even closer relationship to energy-yielding pathways. The occurrence of high concentrations of proline in

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Fig. 2.4. Diagram to illustrate the factors involved in the absorption of food materials from the midgut of the locust. (a) Glucose injected at a concentration of 30 mM; (1), increase in concentration due to absorption of water giving (2) a steep diffusion gradient across the gut wall; absorbed glucose is converted to trehalose (3), and back-diffusion of trehalose (4) restricted by large molecular size and relatively low molarity. (b) Serine injected at a concentration of 30 mM: (1) increase in concentration due to absorption of water, but the diffusion gradient is shallow (2) because of the high haemolymph concentration, and serine is therefore absorbed at a much slower rate than glucose. Data from Treherne (1958) and (1959).

the haemolymph would therefore present no obstacle to the rapid uptake of the bulk of digestive products from the gut.

The absorption of fat has not been investigated so extensively, but it has been shown that here again the midgut caecae are responsible for the bulk of absorption. In the cockroach the rate of uptake is greater with partially hydrolysed material, but total degradation does not appear to be essential for absorption.

5. Nutrition

An intake of carbohydrate, or of protein or of fat, will furnish the metabolic system with its requirement in relation to the provision of energy. Any one of these materials can be oxidized through the Krebs cycle, and a part of their energy trapped in high energy phosphate linkage for subsequent use by the system. But more than energy is required for the maintenance of the metabolic system; enzymes need to be synthesized, co-factors of various kinds must be

made available, and a variety of highly complex molecules must be elaborated to serve as components of intracellular and extracellular architecture. Many of the compounds needed for these purposes can be furnished from simple starting materials through normal pathways of intermediary metabolism, but there is a large number which insects, in common with most other animals, are incapable of synthesizing, and these must be provided ready made as elements of diet.

The need for specific raw materials of this kind is associated with the general turnover of tissue components, which has been shown to characterize living organisms in general, and it is therefore ever present. It becomes acute, however, during periods of growth and during periods of reproduction, particularly in the female who must make the more substantial contribution to the resources of developmental stages. Nutritional requirements are therefore usually assessed on the basis of the capacity of specific diets to maintain growth and reproduction. One of the main difficulties in making accurate assessments on this basis arises from the presence of different sorts of microorganism in the alimentary canal of most insects. As has been mentioned above, these may play a specific part in the digestion of particular types of food, but apart from this they will serve as a source of raw materials of the kind under discussion, by virtue of their ability to synthesize a much greater variety of organic molecules than their hosts. These molecules will become available to the host in the course of the normal turnover of populations of microorganisms, being released by the death and disintegration of organisms within the alimentary canal. It may therefore be possible for an insect to sustain normal growth and reproduction on a diet which in reality is deficient in certain respects, thanks to the supplement derived from its intestinal fauna. To obtain unequivocal evidence of the importance of a given constituent of diet it would be necessary to work with insects which have been deprived artificially of their intestinal microorganisms and to achieve this is usually a matter of considerable technical difficulty. For this reason the number of insects for which nutritional requirements have been firmly established remains small.

Nutritional requirements of three different kinds are usefully distinguished:

(a) the requirement for specific amino acids, which would be needed for the elaboration of enzymes and other proteins. Since proteins are a major constituent of the living organism, the need for raw material in this category is substantial, usually of the order of milligrams per gram dry weight of diet:

(b) the requirement for certain lipids, including unsaturated fatty acids and steroids, which constitute important constituents of cellular membranes. The quantities required are usually much less than a milligram per gram dry weight of diet; and

(c) the requirement for vitamins. Many of these have been identified as co-factors and enzyme components, and are needed, therefore, only in catalytic amounts. Adequate provision for growth is made at a level of micrograms per gram dry weight of diet.

a. Amino Acids

Amino acids are distinguished as essential or non-essential, depending on whether they need to be provided in the diet, or whether they can be synthesized from non-nitrogenous sources. The essential amino acids in insects are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine, the same 10 as in the rat and many other mammals.

Non-essential amino acids include alanine, aspartic acid, glutamic acid and glycine, not surprisingly in view of the fact that the keto-analogues of these amino acids are members of the pathway of carbohydrate degradation (see Chapter 1), so that they can be synthesized from carbohydrate precursors by the simple process of transamination. Proline and hydroxyproline, which can be derived from glutamic acid, also belong to this category, as does serine, which appears to be capable of derivation from glycine. Tyrosine, and the sulphur-containing amino acid cysteine, are not essential provided their essential precursors, phenylalanine and methionine, are present in excess of minimal requirements.

b. Lipids

Insects appear to be unable to synthesize steroids, and they therefore show a specific requirement for cholesterol, or for some suitable precursor in the form of a short-chain derivative like ergosterol, which can substitute in most species.

Cholesterol
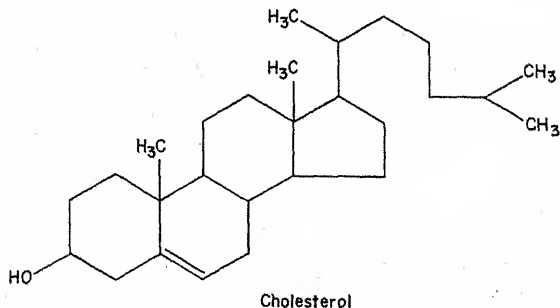
Other derivatives, like stigmasterol or sistosterol, which are characteristic of plant tissues, may satisfy the requirements of phytophagous species, but cannot usually do so in carnivores.

Many insects appear to synthesize all fatty acid requirements from non-lipid sources, and in fact the inclusion of fatty acids in the diet often appears to be detrimental. In a few species, however, a requirement for certain unsaturated fatty acids has been demonstrated (e.g. linoleic acid for Lepidoptera).

c. Vitamins

The study of vitamin requirements is complicated by the fact that such very

small quantities are involved; sufficient amounts may therefore be held in reserve to tide the insect over periods of temporary deprivation, and in some species deficiency symptoms may not appear for several generations. Under such circumstances, the use of antivitamins, substances which inhibit the activities of specific vitamins, may provide more reliable evidence of requirements.

The following vitamins have been found to be essential dietary constituents for all species which it has been possible to study uncontaminated by microorganisms.

(i) Thiamine. A constituent of enzymes involved in carboxylation and decarboxylation.

(ii) Riboflavin. A constituent of the flavoproteins, which play a part in the transport of hydrogen.

(iii) Nicotinic Acid. A constituent of the coenzymes of dehydrogenation.

(iv) Pantothenic Acid. A constituent of coenzyme *A.*

(v) Biotin. The precise nature of its action is obscure, but this compound is thought to be involved in reactions of deamination and decarboxylation.

(vi) Choline. This serves as a donor of methyl groups and is a constituent of acetyl choline and of certain phospholipids.

The following have been found to constitute dietary requirements in most insects, but certain species appear to be capable of growth and reproduction without them.

(i) Pyridoxine. A coenzyme of transamination.

(ii) Folic Acid. A substituted pteridine which serves as a coenzyme in group transfer reactions involving formate.

The following vitamins are apparently synthesized by most species of insect, and are not required as dietary constituents.

(i) Carnitine. A substance involved as a transport factor in the oxidation of fatty acids (certain tenebrionid beetles are exceptional in showing a requirement for this substance).

(ii) Fat-soluble Vitamins. These include vitamins A, D, E and K.

6. Conclusion

The supply of food to the metabolic machinery of insects appears to show few features which could be regarded as typical of the class; within the group there have been many adaptations to different feeding habits, but the general way in which the food materials are manipulated once they have been ingested shows no striking peculiarities, and the requirement for specific items of diet is very similar to that of other animals. The only feature which appears to be specifically related to the insectan organization is the "facilitated" absorption of glucose.

THE CIRCULATORY SYSTEM

When food materials have been absorbed through the walls of the alimentary canal they are transported by the circulatory system to sites of tissue respiration and synthesis, or to sites of food storage. It will therefore be appropriate at this stage to consider the nature of the circulating medium and the mechanism of its circulation. First it should be mentioned that the extracellular fluid under consideration serves not only as a circulating medium, analogous to the blood of higher forms, but also as a bathing medium for the cells of many tissues, analogous to the lymph of vertebrates. By reason of this dual function it has been given the name of haemolymph.

1. The Composition of Haemolymph

a. Soluble Components

The concentration of soluble components in insect haemolymph is extra-ordinarily variable. Freezing point depressions, which provide a convenient measure of total osmotic concentration, range from $0.4°$ to $1.1°$ in different insects, corresponding to a range of between 0.7% and 1.9% sodium chloride equivalents. Differences are substantial not only between species, but between different stages in the life history of a single species, and even within a single stage, depending on variations in physiological state.

Even more striking than the variation in total osmotic concentration is the variation in proportionate composition, examples of which are shown in Fig. 3.1. In many species, particularly among the primitive classes (Apterygota and Exopterygota), inorganic ions make up the bulk of osmotically active material (Fig. 3.1(a)), with sodium as the predominant cation and chloride as the predominant anion. Members of the Phasmidae are peculiar in this respect, with very high concentrations of magnesium and phosphate (Fig. 3.1(b)). In the more advanced classes, among the Endopterygota, inorganic ions constitute a much smaller proportion of the total osmolar concentration (Fig. 3.1(c)), and they make up only about 25% in many butterflies and moths (Fig. 3.1(d)) and even less in certain beetles (Fig. 3.1(e)). In such insects the bulk of osmotic activity is
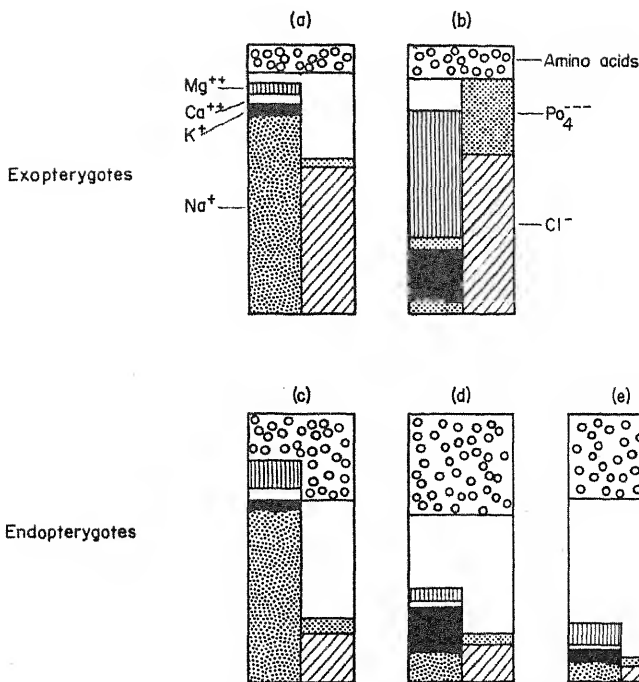
Fig. 3.1. Proportionate ionic composition of the haemolymph of different insects. The blocks represent total osmolar concentration, with relative cation contributions shown on the left and relative anion contributions on the right. The large blank areas represent the proportion accounted for by components of the haemolymph other than those listed (redrawn from Sutcliffe, 1963). (a) Various exopterygote orders (e.g. Odonata, Plecoptera, Heteroptera). (b) Phasmidae. (c) Various endopterygote orders (e.g. Neuroptera, Trichoptera, Diptera). (d) Lepidoptera and Hymenoptera. (e) Certain Coleoptera.

accounted for by amino acids and other organic materials. Here members of the Lepidoptera and Hymenoptera are of interest in having relatively high concentrations of potassium, which often substantially exceed the sodium concentrations (Fig. 3.1(d)). It was thought that this characteristic might be associated with the plant feeding habit which characterizes these classes, since plant material tends to be relatively rich in potassium. But, in fact, many phytophagus species have been shown to have high proportions of sodium, and other factors clearly come into play to determine the sodium/potassium ratio. Whatever their cause, these variations in the proportion of inorganic ions are of considerable interest in relation to the functioning of excitable tissues, and they will be discussed further in that context (see Chapter 8).

In most insects a substantial fraction of the osmotic activity is made up by free amino acids, which occur in concentrations ranging from 200-2000 mg/100 ml. A great deal of work has been done to elucidate

quantitative details of the pattern in different species and these studies have revealed a situation of bewildering complexity. Most amino acids have been shown to be present in all the insects studied, but their relative importance is subject to spectacular variation. Virtually any amino acid may constitute a major element in the haemolymph of one species at some stage in its life history, and be virtually lacking in another species. Part of the recorded variation is probably associated with short-term fluctuations in the concentration of particular amino acids associated with metabolic activities of different kinds. In the silkworm, for instance, the concentration of methionine, glutamate and aspartate fluctuates greatly in the course of larval and pupal development, correlated with changes in the secretory activity of the silk glands. Similarly, in the tsetse fly, the concentration of proline may be extremely high in the resting insect but it decreases greatly during flight, while alanine shows the converse relationship. Instances of this sort suggest that a satisfactory interpretation of the amino acid pattern in insects must await elucidation of the part which different amino acids play in the metabolism of the different species. In the absence of such information, only the broadest and most tentative generalizations can be hazarded, namely:

(a) that certain amino acids including aspartic acid (and its amide asparagine), leucine and isoleucine tend to be poorly represented in the haemolymph of insects; and

(b) that glutamic acid (and its amide, glutamine) and proline are usually well represented in the haemolymph of insects, and often attain levels of concentration equal to or exceeding those of trehalose. It is possible that this should be seen as a reflection of the part played by both of these substances as substrates for flight metabolism as discussed in Chapter 1.

Another major organic constituent of insect haemolymph is trehalose, whose function has already been discussed. In the resting insect concentrations normally range from 500-5000 mg/100 ml depending on the species, thus constituting a substantial fraction of the osmolar concentration. Glucose and other carbohydrates are generally present in very much lower concentration, an exception being the honey bee, where both glucose and fructose concentrations may be high. Another carbohydrate, glycerol, has been demonstrated in extremely high concentration in the haemolymph of a number of species; its occurrence in quantity is usually associated with the development of cold-hardiness, and the subject will be discussed further in Chapter 15.

Other substances which may contribute substantially to osmotic activity in the haemolymph are a variety of organic acids of the Krebs cycle, some of which may attain to concentrations of 200 mg/100 ml, and play a major part in ionic balance, accounting for as much as 40% of cation binding. Organic phosphates, such as α-glyceraldehyde phosphate and glucose-6-phosphate may also be present in quite high concentrations, of the order of 50 mg/100 ml; and nitrogenous

waste products, such as uric acid and allantoin, may contribute to osmotic activity at concentrations of 2-20 mg/100 ml.

In addition to these small organic molecules, insect haemolymph contains considerable amounts of soluble protein, generally in the region of 1000-5000 mg/100 ml. With the development of microtechniques for starch gel electrophoresis and similar fractionation procedures, the haemolymph proteins of a number of species have come under investigation. A large number of components can usually be identified, and spectacular changes have been found to occur at different stages of the life history, with particular protein bands appearing at particular stages of development. The functional significance of these changes have not yet been elucidated, except in so far as some of the bands have been shown to be associated with enzymatic activity. Indeed, haemolymph enzymes appear to constitute a surprisingly high proportion of total protein, and many different kinds of catalytic activity have been shown to be involved, including hydrolysis, dephosphorylation, dehydrogenation, oxidation and transamination.

TABLE 3.1

A comparison between the content of soluble material in the haemolymph of insects and in the blood of vertebrates

Class of substance	mg/100 ml	
	Insect haemolymph	Vertebrate blood
Inorganic	750	850
Organic		
proteins	3000	20,000
carbohydrates	2000	90
amino acids	1200	60
organic acids	100	30
sugar phosphates	50	30
nitrogenous waste	10	30

Data for vertebrate blood from "Biochemists Handbook" (C. Long, ed.). E. and F. N. Spon Ltd., 1961.

Bearing in mind the tremendous variation in composition which characterizes the haemolymph of insects, a rough attempt has been made in Table 3.1 to compare the distribution of soluble constituents in the haemolymph of an endopterygote type of insect with that of vertebrate blood. Apart from the very high protein content of vertebrate blood, associated with the development of a respiratory pigment, the most striking difference concerns the concentrations of carbohydrates and free amino acids, which are about 20 times as great in insect haemolymph as in vertebrate blood. The possible significance of this difference will be discussed in greater detail below.

b. Haemocytes

The haemolymph of insects usually contains a variety of cellular elements, collectively known as haemocytes. They do not make up a constant feature of the circulating medium, since at times most of them may form transient aggregations on organ surfaces, leaving few free in the circulation. In some species the haemocytes may even form stable cell masses in specific regions of the body, apparently playing a part in haemotopoiesis, the formation of blood cells. At other times, particularly after injury or during moulting, the major proportion of haemocytes may circulate freely in the haemolymph, the number of cells reaching levels greater than 50,000 per cubic millimetre.

A number of different types of haemocyte, some of which are illustrated in Fig. 3.2 have been described. They may be distinguished on the basis of their shape, the type of nucleus and the presence or absence of cytoplasmic inclusions. The relationship between the various types of cells has not yet been established unequivocally. They appear to perform a variety of functions including:

(i) Phagocytosis. Certain types of haemocyte are particularly active in the ingestion of particulate material, whether exogenous (insoluble dyes or dead bacilli injected into the haemolymph) or endogenous (fragments of tissue resulting from histolytic activity). In some insects these phagocytic haemocytes may form loose aggregations which serve as haemolymph filters.

(ii) Encapsulation. Haemocytes have been shown to play a part in the encystment of various foreign objects, including metazoan parasites. They form a capsule round the invading organism, and may cause its death by cutting off supplies of food or of oxygen. The encapsulating haemocytes often transform to connective tissue membranes, and a process of melanization sometimes is involved.

(iii) Wound Healing. Haemocytes often tend to aggregate at the site of injury, where they may proliferate to form a plug which helps to seal the wound.

(iv) Coagulation. The haemolymph of different insects appears to differ considerably in its capacity to form a clot. In some species coagulation seems not to occur at all, in others an apparent coagulation may result from an aggregation of haemocytes, while in many a true coagulation of the plasma occurs, involving the precipitation of haemolymph proteins. A special type of "hyaline" haemocyte appears to play a role in plasma precipitation; disruption of these cells in contact with a foreign surface is apparently associated with the release of a factor which promotes coagulation.

(v) Metabolism. Haemocytes appear to play an active part in the intermediary metabolism of insects, as evidenced by the occurrence within them of a variety of stored materials, including glycogen, neutral mucopolysaccharides, phospholipids and proteins. They have been implicated in the formation of basement membranes, of particular hormones, and of melanin.
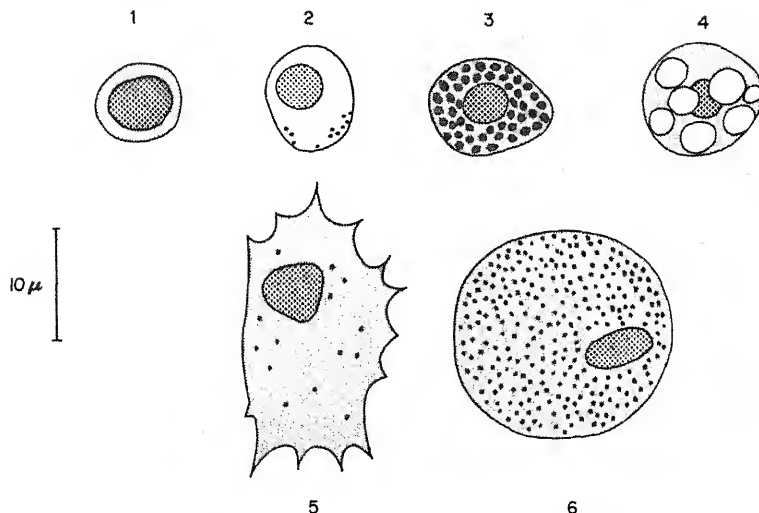
Fig. 3.2. Diagrams of the major types of haemocyte. 1, prohaemocyte, considered to represent the stem cell for other types; 2, coagulocyte, whose disintegration is associated with plasmacoagulation; 3, spherule cell, with large granular inclusions which may contain tyrosinase; 4, adipohaemocyte, with lipid droplets in the cytoplasm; 5, plasmatocyte, a phagocytic haemocyte; 6, oenocytoids, large cells often of irregular shape with granular or crystalloid inclusions (drawn from phase contrast photographs of Jones, 1964).

2. The Pericardial Cells

Before proceeding to a consideration of haemolymph circulation it will be convenient to deal briefly with a type of cell, the so-called nephrocyte, whose functions appear to be essentially similar to those of certain haemocytes. These cells are often closely associated with the dorsal vessel of the circulatory system and under these circumstances they are usually referred to as pericardial cells. They occupy fairly constant stations, as lobes or clusters of cells, on the outer wall of the dorsal vessel or within its lumen. The cells are often filled with red, yellow, green or brown pigments, and their most striking attribute is their ability to take up and store a variety of injected dye materials. It is likely, however that their functions involve more than just a simple segregation of haemolymph contaminants and waste products, especially since the content of such materials shows no progressive increase with age. Like some of the haemocytes, these cells may be active in intermediary metabolism, and they seem also to represent a site of hormone synthesis, as will be shown below.

3. The Circulation of Haemolymph

The haemolymph of insects circulates through a system of sinuses, collectively referred to as the haemocoele, interposed between the various tissues

of the body; these haemolymph channels lack a true endothelial lining, and are often rather poorly defined. The main propulsive force is provided by peristaltic contractions of the dorsal longitudinal vessel; in many species its action is reinforced by the activity of accessory pulsatile organs, and by contractions of dorsal and ventral fibromuscular diaphragms (see Fig. 3.3).
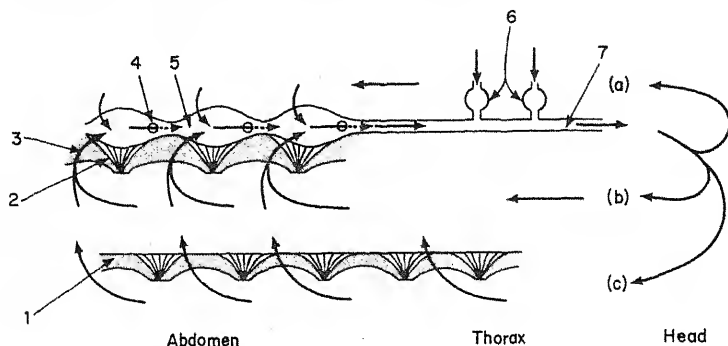
Fig. 3.3. Lateral diagrammatic view of the haemolymph circulation in an insect. 1, ventral diaphragm; 2, alary muscles; 3, dorsal diaphragm; 4, ostium; 5, heart; 6, accessory pulsatile organs facilitating the return of haemolymph from the wings; 7, aorta. (a) pericardial system; (b) perivisceral system; (c) perineural system of haemolymph channels. The heavy arrows indicate the normal course of haemolymph flow (based on Wigglesworth, 1965).

The dorsal vessel is usually a straight tube extending the length of the body, and comprising two main regions:

(a) a posterior "heart" with paired ostia opening into it in each body segment; the ostia are guarded by valves, and the inward projection of these valves may serve to sub-divide the heart into a series of chambers; and

(b) an anterior "aorta" which lacks lateral openings.

The dorsal vessel is suspended from the body wall by elastic filaments, and these serve also to attach it to the dorsal diaphragm when present. This septum underlies the heart, and is usually associated with a series of fan-shaped alary muscles, each converging to an insertion on the lateral body wall. Another diaphragm may be present immediately above the ventral nerve cord. Such transverse membranes tend to divide the system of haemolymph channels into three main regions—a pericardial region surrounding the heart, a perineural region surrounding the ventral nerve cord, and a perivisceral region surrounding the gut (see Fig. 3.3 (a), (b) and (c)). In many species, however, the diaphragm may be extensively fenestrated and would then provide only a partial barrier to the exchange of haemolymph between regions.

A number of accessory pulsatile organs have been described in different insects. They are situated usually at the base of antennae, wings (see Fig. 3.3) or

legs, and serve to promote the movement of haemolymph in the corresponding appendages remote from the main pathways of circulation.

Peristaltic contractions of muscles in the wall of the longitudinal vessel, usually initiated at the posterior end and progressing towards the head, serve to force blood into the aorta, from which it is discharged into the anterior sinus systems. From here the blood percolates backwards between the tissues of the body, and is ultimately drawn back into the posterior parts of the dorsal vessel by the fall in pressure associated with its relaxation. The sequence of mechanical events during a cycle of contraction is illustrated in Fig. 3.4(a), which shows a
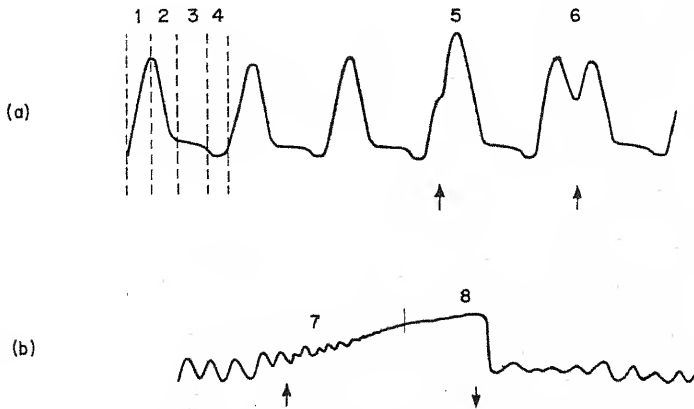
Fig. 3.4. Records of the mechanical activity of the cockroach heart. (a) Normal heart beat, showing systole (1), diastole (2), diastasis (3) and "pre-systolic notch" (4); at (5) the heart was stimulated with a single shock during systole, as marked by the arrow, and at (6) the stimulus was delivered during diastole. (b) The effect of stimulation at moderate frequency, starting at the first arrow, is to produce progressive summation (7) leading eventually to tetanus (8). Jones, 1964 based on Yeager.

phase of contraction (systole), a phase of relaxation (diastole) and a phase of diastasis, the vessel pausing for a time in the relaxed condition. The slight presystolic notch is thought to reflect a passive expansion of the vessel associated with the build-up of pressure during the contraction of adjacent regions. The heart appears to remain excitable at all stages of the contraction cycle, the refractoriness which is so characteristic of the vertebrate heart being completely absent. Electrical stimulation in early systole leads to summation of the induced contraction, and in diastole or diastasis it produces an early contraction (Fig. 3.4(a)). In fact, it is possible to tetanize the heart of a cockroach, with normal rhythmicity completely abolished (Fig. 3.4(b)). The mechanical activity is associated with complex changes in electrical potential, comprising partially fused fast diphasic and slower monophasic waves.

The rhythmic contraction of the dorsal vessel appears to be myogenic in

many insects, and fragments of vessel completely isolated from the body may continue to beat, though at a much slower rate than normal. Although the beat may be initiated inherently, it is subject to control by nervous and humoral influences. A pair of lateral nerves accompany the dorsal vessel, made up of segmental branches from the ventral ganglia and from the cardiac ganglia of the stomatogastric system, and the modifying influence of this innervation has been demonstrated by electrical stimulation of associated parts of the nervous system, which may cause arrest of heart beat, or acceleration of the beat frequency, depending on the rate of stimulation.

The action of a variety of pharmacologically active substances has been investigated with various insect heart preparations. Acetylcholine appears to have a stimulating effect on heart rate, an effect which can be blocked by atropine and curare, and potentiated by eserine, suggesting that cholinergic pacemakers may be involved. The effects of other drugs, like nicotine and adrenalin, on isolated heart preparations are rather variable and difficult to interpret in the present state of knowledge. There can, however, be little doubt that humoral control is of importance, in view of the isolation from the corpora cardiaca of substances capable of causing a marked acceleration of the beat frequency in semi-isolated preparations. The active principle has been partially purified, and appears to be a peptide; its action is apparently an indirect one, in that it causes the release from pericardial cells of a cardiac stimulator. The increased rate of heart beat which occurs after feeding in a number of insects is perhaps the outcome of activity in this chain of effectors.

4. Conclusion

This brief review of the insect circulatory system has focussed attention on the simpler and more regular aspects of its activity, and may therefore have left an impression of a fairly well organized system providing for the efficient transport of materials from one part of the insect's body to another. The reality of the situation is probably quite different, and it may be well to list a few of the more startling observations concerning the aberrancy of the physiology of circulation, to serve as a corrective against too superficial a view. It has, for instance, been shown that the heart can be removed completely from some insects without causing their death; and in many, the heart may stop for long periods without death or "evident dismay" on the part of the insect. Reversal of the direction of beat is commonly observed in many different species, and in most the rate of beat may vary between very wide limits. The flow of haemolymph in the open system of sinuses shows corresponding irregularities, sometimes moving in jerks synchronous with contractions of the dorsal vessel; often it is slow and discontinuous and in some regions of the body it may cease altogether for long periods. Experiments involving the injection of dyes or of

radioactive material into the haemocoele have shown that the mixing of haemolymph is relatively inefficient, and it may take as long as 15 min for injected material to move from a superficial sinus to the dorsal vessel. Observations of this kind suggest that the analogy between the circulatory system of vertebrates and that of insects should not be pushed too far; although they may have many functions in common the parallel appears to break down in a number of cases, and it seems likely that this may be associated with corresponding divergences at the level of circulatory mechanism.

One of the most striking differences between the circulating medium of insects and of vertebrates is, of course, that in vertebrates it serves as a carrier of oxygen, while in insects, with their extensive tracheal system (see Chapter 7) the haemolymph would play a marginal role in oxygen transport. This difference has enormous implications in terms of functional organization; in vertebrates the demand for oxygen by every cell in the body is urgent and constant, and the system responsible for its transport must show a corresponding level of efficiency; where respiratory requirements are met by a tracheal system, the transport function of blood may be imagined to become much less demanding, since it will involve materials like carbohydrates which can be carried in quite concentrated solution, or materials like hormones, where fast supply is not a primary requirement. Under these circumstances one could imagine that the selection for improvements in the system as a transport system might become subordinate to selection for other aspects of function. Of these, the most important might be the function of haemolymph as a store, both of water and of dissolved materials of various kinds. The importance of this function is clearly indicated by the high concentration of soluble materials, and by the no less striking changes in haemolymph volume, which are very difficult to reconcile with the notion of an efficient transport system. It is common knowledge that it is possible to draw quite large quantities of haemolymph from many species of insect, provided they are in the appropriate physiological state, while at other times and in other species the insect may appear almost dry, and the quantity of haemolymph is clearly exceedingly small. Haemolymph volumes have been determined for a number of different species, and values ranging between 0.9% and 45.4% of the total body weight have been reported. Wide variations occur within a species depending on the stage of development and general physio-logical condition. The changes associated with ecdysis are particularly striking, as shown in Fig. 3.5. There can be little doubt that the marked rise in haemolymph volume which occurs at the time of moulting is associated with the function of haemolymph in providing for the hydrostatic distribution of pressure to ensure the proper expansion of the cuticle, a process which will be discussed further in Chapter 13. Such changes in blood volume have been shown to be in part attributable to a shift of fluids between haemocoele and intracellular reservoirs, and they are likely to become of particular importance during periods of
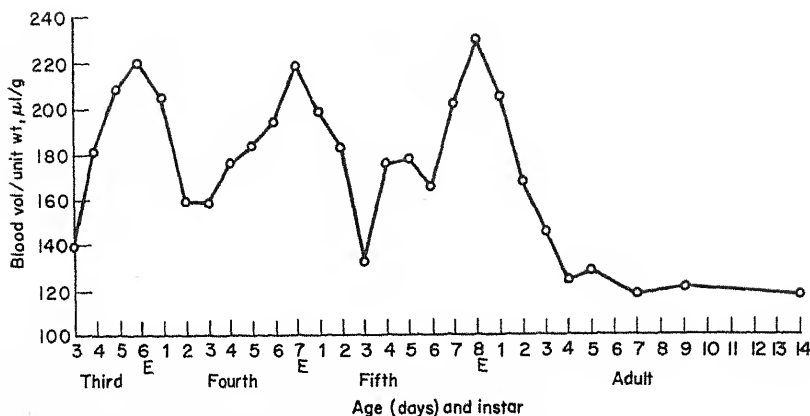
Fig. 3.5. Variations in the volume of blood per unit weight of insect during the development of the locust from the third instar. E denotes the time of ecdysis (Lee, 1961).

desiccation, when the haemolymph could act as a reserve of water for the tissues.

Once it is appreciated that functions of the haemolymph other than transport may be of paramount importance, the apparent inefficiency of the circulatory system in a class of animals which otherwise embodies so many elegant adaptations ceases to be an embarrassment, and certain general features of insect organization may then be seen as bound up with the problems that would arise when transport function is made subsidiary. The tendency for the different organ systems of insects to be extraordinarily diffuse, compared with their counterparts in higher animals, is an example. The tracheal system of necessity ramifies to all parts of the insect body, but the fat body, too, considered by some as in certain respects analogous to the liver of higher animals, is remarkably diffuse, and there are few parts of the insect's body which are far removed from cells of this tissue. The excretory organs, also, tend to be distributed widely, with tubules of the Malpighian system ramifying extensively in the abdomen, and extending into the thorax in many species. It seems not unlikely that such a broadcasting of the main organ systems may be related to the inefficiency of circulation, to the likelihood that stagnant pools may form in the haemocoele, and that such pools would have to rely on diffusion rather than on bulk flow for the purging of toxic products and for the replenishment of reserves; the proximity of an excretory tubule to such a toxic pool, or of a collection of fat body cells, would ensure that the path of diffusion remains a short one, and the process of diffusion therefore relatively efficient. The high concentration of many metabolites in the haemolymph is of obvious relevance in this context, since this would serve to maintain a steep diffusion gradient between

haemolymph and site of metabolism, and the occurrence of a variety of enzymes in the haemolymph may also be pertinent, serving to transform substances diffusing into the haemolymph, and thus to maintain the diffusion gradient. It may even be that one of the basic insect characteristics, namely small size, should be seen in part as a reflection of circulatory inefficiency; for it is likely that only an animal of relatively small dimensions could rely as extensively as an insect appears to do on the process of liquid diffusion, for the satisfaction of many of its somatic requirements.

FOOD STORAGE

The products of digestion, following their absorption from the alimentary canal, may be used directly to satisfy metabolic requirements, or they may be deposited in food stores from which they can be drawn in times of need. The cells of a variety of tissues (e.g. muscle cells and cells of the midgut) may be involved in food storage, and the haemolymph itself has been shown to constitute an important reserve of organic materials, but the main storage organ of insects is the fat body. The most important storage materials are glycogen and fat, and some indication of the extent of fat body development is given by the fact that, in some insects, the amount of fat may exceed 50% of the total dry weight of the animal, while glycogen contents in excess of 33% of dry weight have been reported.

The fat body consists of aggregates of cells forming lobes and sheets of tissue, which invest the internal organs of the body and constitute a conspicuous element of anatomy in the well-fed insect. The diffuse arrangement of adipose tissue is such as to facilitate exchange of material between it and the haemolymph which bathes it. The cells of which it is composed are known as trophocytes, and during periods of food intake they grow in size and become filled with droplets of fat and protein, and with particles of glycogen (see Fig. 4.1 (a), (b)). In the well-fed insect the droplets of fat tend to dominate the appearance of the fat body cells; mitochondria are distributed widely and parts of the cytoplasm show a well-developed endoplasmic reticulum bearing ribosomes, to give the characteristic "rough surface" appearance. The regions which are free of endoplasmic reticulum are packed with minute granules of glycogen.

During periods of food deprivation there is a gradual reduction in the amount of stored material; glycogen granules become less numerous and the fat droplets disappear. With prolonged starvation, degenerative changes set in, and the so-called albuminoid granules make their appearance. These are thought to represent remnants of cell organelles undergoing degenerative changes as a result of lysosome activity, the setting up of special regions of the cytoplasm to promote histolysis of intracellular material. There thus appears to be a general
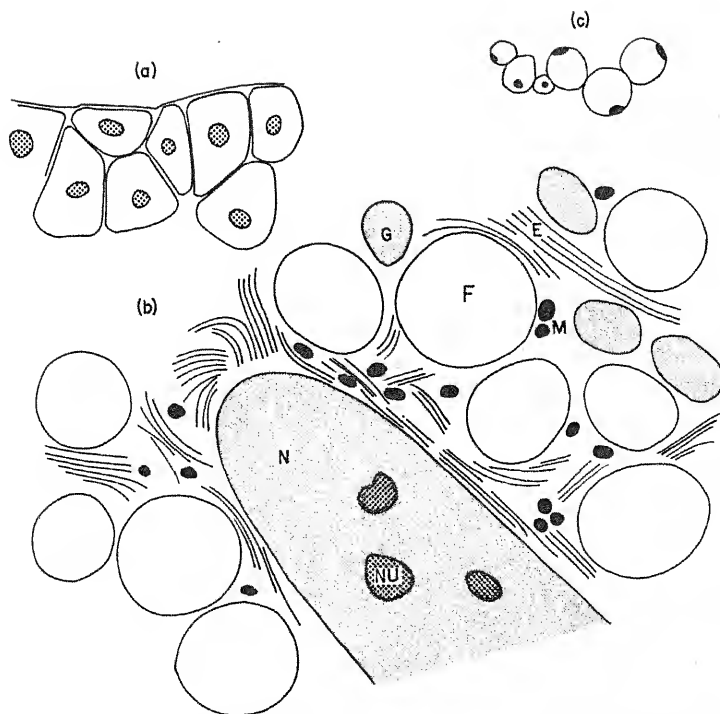
Fig. 4.1. The structure of fat body. (a) General arrangement of cells in the fat body. (b) Diagram of a cross-section of part of a fat body cell. N, nucleus; NU, nucleolus; F, fat droplet; G, glycogen-rich granule; M, mitochondria; E, endoplasmic reticulum (drawn from electron micrograph of Ishizaki, 1965). (c) A cluster of fat droplets from the fat body of an insect, each with its "cap" of lipase, as revealed by the 5-bromoindoxyl acetate method (drawn from photomicrograph of Wigglesworth, 1958).

erosion of the very fabric of the fat body, and the materials which are in this way released presumably serve to buttress other aspects of the insect's metabolism.

There can be little doubt that cyclical changes of a less extreme nature are a feature of the day-to-day existence of many insects. These have not been studied at the level of cellular ultrastructure, but they find reflection in periodic fluctuations in the quantity of reserve. These range from long-term changes, such as the gradual build-up and decline of fat reserves which take place during the life of a tsetse fly (Fig. 4.2(a)); though shorter cyclical changes in fat content, such as have been observed particularly in blood-sucking insects (Fig. 4.2(b)), but would be associated with intermittent feeding or diurnal fluctuations in feeding intensity in many other species; to the quite rapid depletion of glycogen and fat reserves which have been demonstrated for fruit flies and
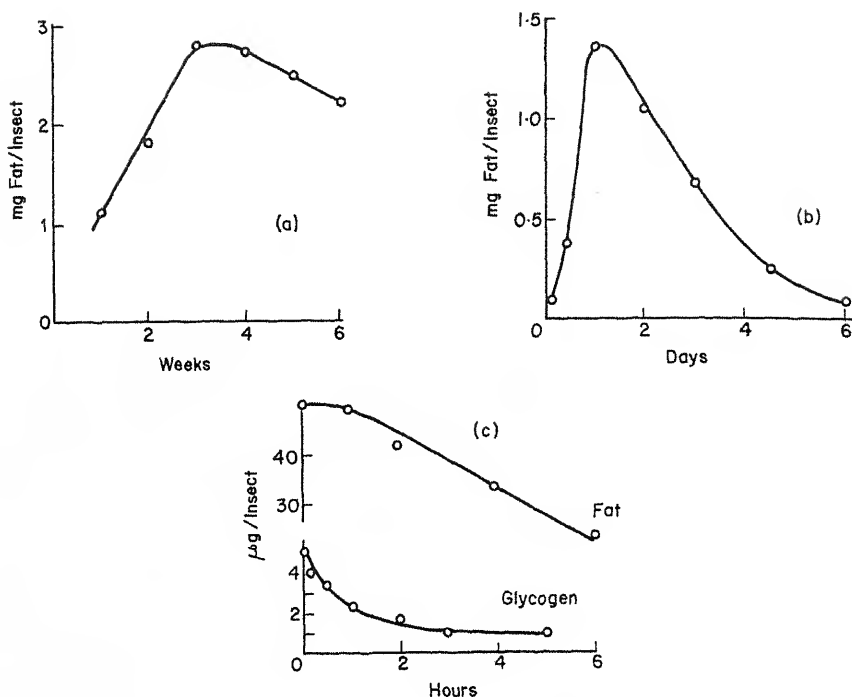
Fig. 4.2. Periodic changes in the quantity of food reserves in insects. (a) The average fat content of a population of tsetse flies in their natural environment, during successive weeks of adult life (from Jackson, 1946); the curve shows a general trend on which day-to-day fluctuations of individual flies (as shown in (b)) would be superimposed. (b) Fluctuations in the fat content of tsetse flies during the course of a single hunger cycle following a blood meal on Day 0 (from Bursell, 1963). (c) Changes in the fat and glycogen content of aphids during successive hours of flight (data from Cockbain, 1961).

aphids during flight (Fig. 4.2(c)); in these insects glycogen is used predominantly during early phases of flight, while fat becomes the principal substrate later.

Long-term changes in the quantity of stored food materials occur also during development, where insects may be deprived of the opportunity to feed for extended periods. In the mealworm beetle, and in the silkworm, a great deal of the glycogen laid down during late stages of larval life is utilized for pupal development; in the tsetse fly metamorphosis appears to be sustained almost entirely by fat reserves, which become greatly depleted in the course of pupal development.

Granted that cells of the fat body perform the function of storage organs for the main classes of food substance—carbohydrates, fats and proteins—the question arises whether the fat cells are themselves responsible for the synthesis of these storage materials from their constituent components—monosaccharides,

fatty acids and amino acids—or whether the storage compounds are wholly or partially synthesized elsewhere and merely taken up for storage by the fat cells.

As far as carbohydrates are concerned, there is evidence that enzymes capable of mediating the synthesis of glycogen from glucose are present in the fat body and also that the fat body is closely associated with the metabolism of trehalose. Radioactive glucose has been found to be converted quickly to trehalose by fat body *in vitro,* while other tissues, such as muscle, blood and gut were found to be relatively inactive. The enzyme trehalase has also been demonstrated in fat body, but its activity there is rather slight compared with other tissues, and it would seem that carbohydrate is mobilized from the fat body as trehalose, and that it is transported in this form to the site of respiration.

The stored fats are present mainly as neutral triglycerides, although free fatty acids may make some contribution to the total. Evidence has recently become available for the existence of an enzyme system in insect fat body capable of synthesizing free fatty acids. Lipases, responsible for the hydrolysis of glycerides to their constituent fatty acids and glycerol have also been shown to be highly active in fat body homogenates, and it has been possible to demonstrate the presence of a small "cap" of lipase associated with each intracellular droplet of fat (see Fig. 4.1(c)). It seems likely, therefore, that fat is not only stored, but also synthesized by the fat body, and that its mobilization from the fat body involves the liberation of free fatty acids from their neutral fats, such fatty acids being transported to respiring tissues for further breakdown.

The situation as far as protein is concerned is not altogether clear. The ability of fat body cells to synthesize proteins, associated with the presence of a "rough" endoplasmic reticulum, has been demonstrated convincingly in studies involving haemolymph proteins. Various amino acids have been shown to become incorporated into proteins which are subsequently released from the fat body into the haemolymph. But the precise nature of the proteins which can be shown by histochemical techniques to be stored in intracellular droplets of the fat body cells remains obscure, as does the mechanism of their mobilization.

In addition to the storage of food materials, certain cells of the fat body may serve as stores of uric acid. The extent to which this occurs differs from species to species, but the phenomenon would appear to be particularly common among the Orthoptera. In certain cockroaches, placed on a diet rich in nitrogen, the fat body may become greatly enlarged through the distension of its cells with white deposits of uric acid. Homogenates of fat body have been shown to be active in the synthesis of uric acid, but the significance of its accumulation in fat body cells is not yet clear. It seems likely that it represents a process of storage excretion, and it will be discussed further in the section dealing with nitrogenous waste (Chapter 6). On the other hand, some authors have suggested that it may represent a storage of nitrogen, which could be mobilized for synthesis of nitrogen-containing compounds during periods of nitrogen deprivation. This is

an attractive idea, but as yet there is no evidence that uric acid is in fact broken down in such a way as to make its nitrogen available for such a purpose.

Apart from their function as a store of the three main types of food material, the fat body has been shown to perform an extremely important function in intermediary metabolism. Its potentiality in this respect has been under intensive investigation during the past few decades, and an impressive spectrum of functional activity has been demonstrated, based on the presence of many different kinds of enzymes including oxidases, dehydrogenases, transaminases, esterases and phosphatases. The special metabolism of purines and certain eye pigments appears to be the particular province of the fat body.

In view of its importance in intermediary metabolism, of its role as a store of food reserves and of the part that it plays in detoxication, it is not surprising that the fat body has been regarded as the insect equivalent of the vertebrate liver.

OSMOREGULATION

The composition of insect haemolymph has been discussed in an earlier chapter, and it was noted that the osmotic concentration differed from species to species, and that different patterns of ionic composition characterized different groups of insects. In some, sodium predominates among the cations, in others potassium, while in the Phasmidae, magnesium is present in higher concentration than the other cations; and similar, though less extreme, variations occur among anions. It would seem that for a given species of insect, at a given stage in its life history, there is a general level of total osmotic concentration, and a general pattern of proportionate composition, and that these serve as the basis for normal physiological function. The question then arises as to the mechanism by which the haemolymph composition is maintained at this appropriate level.

1. The Nature of the Problem

For an insect, as for any other terrestrial animal, there are two main factors which would tend to upset the osmotic and ionic equilibria:

(a) losses of water by transpiration, and gain of water by drinking would tend to concentrate or dilute the body fluids, and lead to a change in osmotic pressure without affecting proportionate composition; and

(b) intake of inorganic salts with the food would lead to an upset of osmotic balance if the osmotic concentration of the food is different from that of the haemolymph, and to ionic imbalance if the ionic composition of the food is different from that of the haemolymph.

It is possible, therefore, to distinguish two different aspects of regulation: the ability to regulate total osmotic pressure, and the ability to regulate ionic composition. In terms of mechanism, however, both depend on the capacity for active transport of solutes, and perhaps of water, on the part of certain specialized epithelia, and the distinction will not be maintained in the discussion that follows.

The problem of osmoregulation in terrestrial insects is thus essentially that of getting rid of such water and salts as may be ingested in excess of requirements arising as a result of losses of water by transpiration and excretion, and of salts by excretion. As far as the salts are concerned, one way, theoretically, of maintaining regulation would be to limit the absorption of salts from the alimentary canal—to take up mainly the necessary organic constituents and to allow most of the inorganic material to pass through as faecal matter. In fact, inorganic ions are quickly absorbed from the alimentary canal, ingested salts therefore mix with the general pool of inorganic material in the haemolymph, and the problem arises of sequestering them in some way from this pool. This becomes a particularly difficult task where the proportion of different ions in the food differs substantially from that which characterizes the haemolymph, and this is often the case, as shown by the values listed in Table 5.1. A primary

TABLE 5.1

The cation composition of the haemolymph of some terrestrial insects compared with that of their food

	Concentration m.eq/l or m.eq/kg wet wt.			
	Na^+	K^+	Ca^{++}	Mg^{++}
Gastrophilus intestinalis (larva)	175	12	6	32
Whole horse blood	85	31	2	3
Dixippus morosus (adult)	9	28	16	142
Privet leaves	46	152	825	40
Leptinotarsa decemmineata (adult)	4	65	48	189
Potato leaves	trace	145	129	86

Data for *Gastrophilus* from Levenbook (1950); otherwise Duchâteau *et al.* (1953).

requirement for sequestration would be the production of a urine, which could serve as a vehicle for the removal of inorganic constituents, and provision would then have to be made for the resorption from this urine of water and of such constituents as would be required to maintain the normal composition of the haemolymph. In insects the Malpighian tubules serve in the formation of urine, while the rectal glands of the hindgut are active in the resorption of wanted constituents, and it will be useful to defer consideration of physiological mechanisms until an account has been given of the structure of these main elements of the excretory system.

2. Structure of the Excretory System

A generalized diagram of the excretory system of an insect is shown in Fig. 5.1(a). The excretory, or Malpighian, tubules lie free in the haemolymph of the abdomen, ramifying among the other abdominal organs. They are closed distally, and open proximally to the alimentary canal at the junction of midgut and hindgut. The number of tubules varies between species, from two to several hundred, and there may be some histological differentiation between tubules, or between different regions of a single tubule. The functional significance of such differentiation has not yet been fully elucidated, and it will not be described in detail. The tubules are lined with a single layer of epithelial cells supported by a conspicuous basement membrane, and associated with each tubule is a spiral band of muscle cells, whose contractions cause writhing movements of the tubules, which may assist in the bulk flow of urine; they are often accompanied along their length by tracheoles.

The epithelial cells of the Malpighian tubules are characterized by a "brush border", and electron micrographs show that the inner surface is extensively folded to produce a layer of tightly packed microvilli, many of which contain elongated mitochondria (see Fig. 5.1(b)). The basal plasmalemma is also extensively folded, and these invaginations, too, are associated with a high density of mitochondria. The submicroscopic structure thus conforms to the pattern of cells which are engaged in intense secretory activity.

The contents of the Malpighian tubules enter the alimentary canal to mix with end-products of digestion from the midgut, and pass backwards through the hindgut to the rectum. The epithelial cells of the hindgut and rectum are covered by a distinct cuticular lining, and are invested by incomplete layers of muscle, whose contractions cause deformations of the rectal chamber, and thus efficient mixing of the rectal contents. A conspicuous feature of the rectal anatomy of many insects are the so-called rectal glands, whose precise form varies from species to species. In some they are represented by longitudinal bands of well-developed glandular cells (Fig. 5.1(c)); in others they may form distinct papillae, projecting into the rectal cavity (Fig. 5.1(a)); in all species, even those that show no marked differentiation between glandular patches and the general rectal epithelium, the organs are richly supplied with tracheoles.

3. The Production of Urine

The primary requisite for ionic regulation in terrestrial animals is the formation of urine. In view of the low-pressure, open and often erratic nature of the blood circulation in insects (see Chapter 3), the possibility of using some form of filtration system as a basis for urine formation, as in the vertebrate kidney, is clearly excluded, and recourse must be had to a quite different
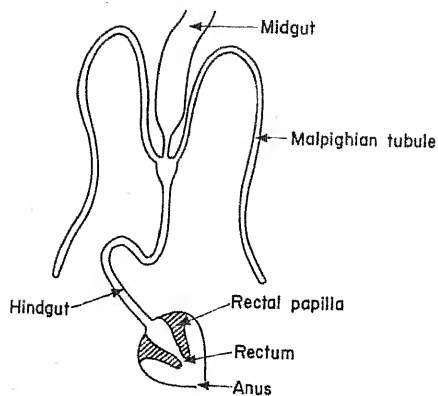
(a)
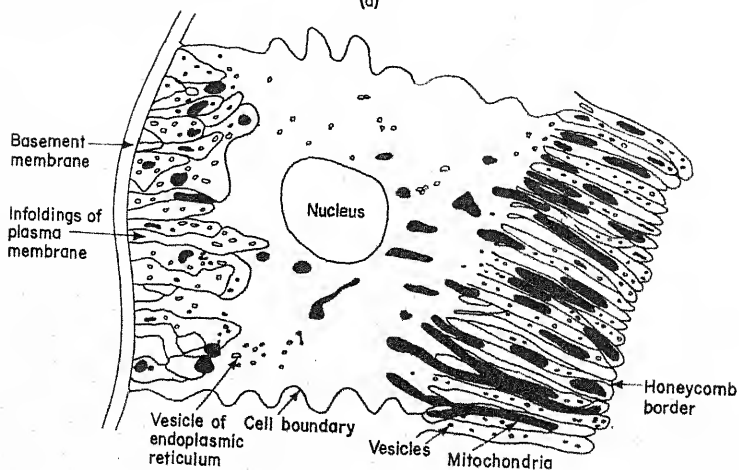
(b)
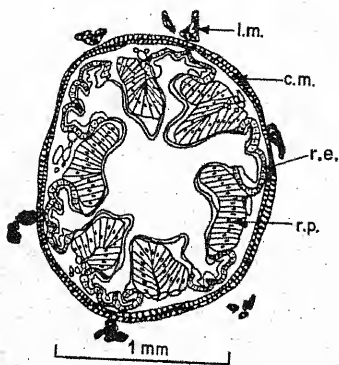
(c)

principle. It has in fact been demonstrated that the urine of insects is formed by an active secretion of inorganic ions, potassium in particular. This was brilliantly established by the work of Ramsay, who developed techniques for the collection and analysis of minute quantities of fluid from different parts of the excretory system of a number of different species of insect, and his results have been fully confirmed by later workers. Figure 5.2(i) shows one of the simple yet delicate systems employed by Ramsay in his original investigation. Segments of Malpighian tubule were isolated in a drop of haemolymph under liquid paraffin, with an adequate supply of oxygen assured. The severed end of the tubule was drawn out of the drop of haemolymph by means of a fine silken thread, and a cut was made in the tubule close to the silk ligature, allowing the urine produced to escape as a droplet round the ligature, from which it could be collected for analysis. It was found to be approximately isosmotic with the haemolymph, but its potassium concentration was very much greater (see Table 5.2). This inequality in the distribution of potassium between haemolymph and tubule could not be accounted for on the basis of an electrical gradient across the tubule wall; in *Dixippus*, for example, the tubule content is positive with respect to haemolymph, which would tend to oppose an inward transfer of potassium; and even in insects where the tubule interior carries a negative charge (as in the locust and in *Rhodnius*), the magnitude of the potential difference is not commensurate with the difference in potassium concentration. It would seem, therefore, that potassium is transported from the haemolymph across the tubule epithelium and into the lumen against an electrochemical gradient, and that the process must therefore involve some active mechanism. The potassium ions are probably accompanied by chloride to preserve the electrical equilibrium, so that what is effectively involved is a transport of neutral salt across the tubule epithelium. The effect of such an active transport would be to increase the osmotic pressure of the tubule fluid, yet its osmotic concentration does not differ greatly from that of haemolymph (see Table 5.2). This shows that the active uptake of salts is associated with an isosmotic uptake of water; this, in turn, would increase the hydrostatic pressure inside the tubule and cause a bulk flow of fluid along it, in other words, a flow of urine.

Further work on isolated Malpighian tubules has amply confirmed this concept of urine formation. The special role of potassium has been substantiated by investigations of the effect of ionic composition of the medium bathing the tubules on the rate of urine production, and on the composition of urine. As

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Fig. 5.1. Features of the excretory system of insects. (a) Diagram of the interrelationship between the alimentary canal and the excretory system in an insect. (b) Section through a cell of the distal region of the Malpighian tubule of *Rhodnius* (Stobbart and Shaw, 1964, from Wigglesworth and Salpeter). (c) Diagram of a cross-section of the locust rectum. c.m., circular muscle; l.m., longitudinal muscle; r.e., reduced epithelium between rectal pads; r.p., rectal pad (Phillips, 1964).

(i)

(ii)

(iii)

Sucrose concentration in medium (mM/l)
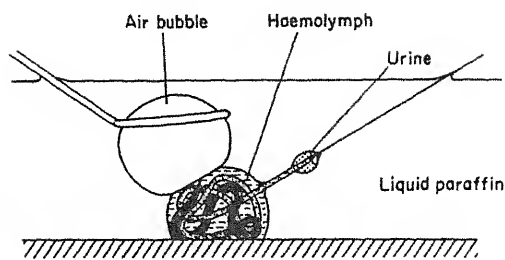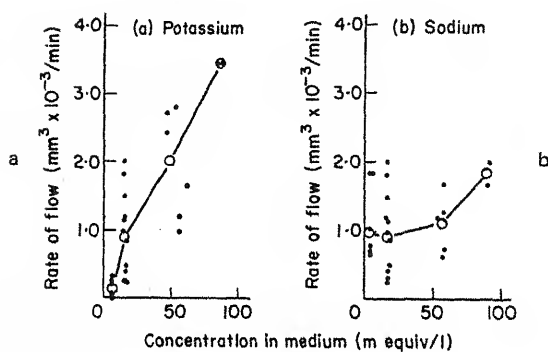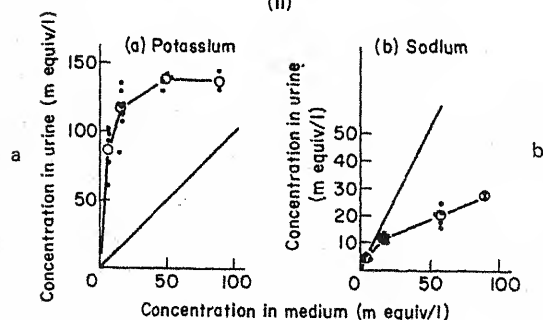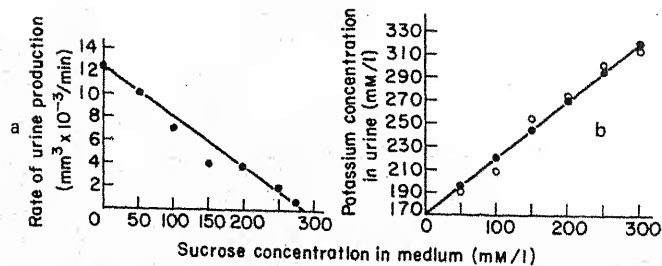
(iv)   (v)

shown in Fig. 5.2(ii), the rate of urine flow is closely dependent on the potassium concentration of the medium, while it is relatively unaffected by changes in the concentration of sodium; and Fig. 5.2(iii) shows that while the potassium concentration of the urine increases sharply with an increase in haemolymph potassium, the concentration of sodium is relatively unaffected by changes in the sodium content of the medium. Recently it has been shown that urine production is also sensitive to the osmotic pressure of the medium (Fig. 5.2(iv)), and that maintenance of urine flow in media of high osmotic pressure is associated with a corresponding increase in the potassium concentration of the urine (Fig. 5.2(v)).

The precise mechanism by which the transport of potassium and water across the tubule epithelium is achieved has not yet been fully elucidated, but it is considered that the complex infoldings of both basal and apical membranes (see Fig. 5.1(b)) are of significance in enabling the setting up of standing osmotic gradients. Ions which have been actively transported into such submicroscopic reservoirs would be prevented from diffusing away by structural barriers, thus enabling water to follow along the osmotic gradients.

With regard to ions other than potassium, and to organic molecules such as amino acids and carbohydrates, consideration of the corresponding electro-chemical gradients suggest that these enter the tubules by passive diffusion from the haemolymph. The most important exception is uric acid, which appears to be actively secreted into the tubule against a steep concentration gradient (see Table 5.2), probably as the soluble sodium or potassium salt.

4. Rectal Resorption

With the formation of a urine containing a variety of inorganic ions which have entered the Malpighian fluid by secretion and by passive diffusion, the indispensable basis for ionic regulation has been secured, but without further manipulation of the urine so produced the sole ionic effect of the process would be a progressive potassium impoverishment. A mechanism enabling selective

Fig. 5.2. Aspects of the physiology of excretion in insects. (i) Isolated Malpighian tubule preparation, see text for explanation (Ramsay, 1954). (ii) (a) The rate of urine flow in the stick insect as a function of the potassium concentration of the medium (Na^+ at 16-17 m.eq/litre). (b) The rate of urine flow as a function of sodium concentration of the medium (K^+ at 15-16 m.eq/litre) (Ramsay, 1955). (iii) (a) Potassium concentration in the urine of the stick insect as a function of potassium concentration in the medium (Na^+ at 16-17 m.eq/litre). (b) Sodium concentration in the urine as a function of the sodium concentration in the medium (K^+ at 15-16 m.eq/litre): the straight lines indicate equal concentration in urine and medium (Ramsay, 1955). (iv) The effect of sucrose concentration in the medium on the rate of urine flow in the blowfly (Berridge, 1968). (v) The effect of sucrose concentration in the medium on the potassium concentration in the urine of the blowfly (Berridge, 1968).

TABLE 5.2

Ionic composition of the haemolymph, urine and rectal contents of the stick insect and the locust

Source of fluid	Osmotic pressure NaCl eq	Uric acid mg/100 ml	Concentration m.eq/l							Potential difference mV
			Na⁺	K⁺	Cl⁻	Ca⁺⁺	Mg⁺⁺	PO̿₄	
Dixippus morosus										
Haemolymph*	171	5	11	18	57	7	108	39 ⎫	+ 21
Urine	171	43	5	145	65	2	18	51 ⎭	
Rectal contents	390	–	18	327	–	–	–	–	
Schistocerca gregaria										
Haemolymph	214	–	108	11	115	–	–	–	
Urine	226	–	20	139	93	–	–	–	
Rectal contents (water fed)	433	–	1	22	5	–	–	–	
Ditto (saline fed)	989	–	405	241	659	–	–	–	

Data for *Dixippus* from Ramsay (1953 and 1955); data for *Schistocerca* from Phillips (1964).
* Values are for the "serum" separated by centrifugation from the clot formed by heating the haemolymph to 100° for 5 min.

resorption of urine components needs to be superimposed in order to achieve ionic regulation, and such a mechanism is provided by the activity of the rectal glands, or the rectal epithelium in insects which lack distinct glands.

The important part which the rectal epithelium plays in ionic regulation is witnessed by the changes in composition which occur after the urine has entered the rectum (see Table 5.2). The total osmotic concentration shows a substantial increase, but the extent to which this is reflected in the concentration of inorganic ions depends on ionic balance. In starved animals which have access to water, the need to conserve inorganic salts would be paramount, and the concentration of sodium, potassium and chloride in the rectal fluid is reduced to extremely low values. The high osmotic pressure of the rectal contents of the locust in these circumstances must then be attributed to organic solutes that in the absence of specific information, may be classed as waste products. In feeding insects, or in saline-fed insects, on the other hand, there would be a need to eliminate excess ions, and their concentration in the rectal fluid remains at a correspondingly high level, accounting for a substantial proportion of the total osmotic pressure.

These changes in composition suggest that the rectum is active in the resorption both of water and of inorganic salts, and that it is here that the composition of the urine is accurately adjusted to make its excretion effective in ionic regulation. The mechanisms involved in rectal function have been investigated by Phillips, whose work will form the basis of the account that follows. The uptake of water and salts by rectal glands of the locust was determined following injection of appropriate solutions into the rectum, separated from the rest of the alimentary canal by ligatures at the junction of midgut and hindgut. Radioactively labelled serum albumin, a substance which is not taken up by the rectal epithelium and cannot diffuse across it, was incorporated in the injection fluids to provide a convenient check on changes in liquid volume associated with the uptake of water.

a. Resorption of Water from the Rectum

When a solution of a carbohydrate which is not absorbed, such as xylose or trehalose, is injected into the rectum of a fasting locust, water is resorbed from the solution even though its osmotic pressure may be substantially above that of the haemolymph. This absorption of water against a concentration gradient is not associated with a significant net uptake of inorganic salts, which raises the possibility that an active transport of water may be involved. It is possible, however, that at the submicroscopic level the movement of water may be an isosmotic one, associated, perhaps, with a cycling of potassium ions across subcellular membranes. Whatever the precise mechanism, the uptake has been shown to be limited by the osmotic gradient, as illustrated in Fig. 5.3(a). With dilute solutions, where the osmotic gradient between rectal contents and

haemolymph would favour an uptake of water from the rectum, absorption takes place at the rate of about 40 μl/hr. In the absence of a favourable osmotic gradient the rate of uptake is substantially reduced, but it is not until the osmotic concentration of the rectal contents greatly exceeds that of the haemolymph that net uptake ceases altogether; and what is of particular interest in relation to the general problem of osmoregulation and water balance is, that the level to which osmotic concentration of the rectal contents can be raised by absorption of water varies according to the water requirements of the insect. In starved individuals with access to fresh water, the resorption of water ceases when the osmotic pressure reaches a value equivalent to a freezing point depression of 1.5°, while in saline-fed insects freezing point depressions approaching 3.0° have been recorded.

b. Resorption of Solutes from the Rectum

To study the absorption of inorganic ions from the ligated rectum of the locust, injection fluids made hyperosmotic with xylose were used, in order to avoid complications which might arise from a simultaneous absorption of water. In this way it was shown that an uptake of sodium, potassium and chloride can occur against a strong electrochemical gradient, and that in water-fed insects the end result is the all but complete removal of these ions from the rectum (see Table 5.2). The rate of absorption depends upon rectal concentration and potassium is taken up very much faster than sodium (Fig. 5.3(b)). The rate of chloride uptake is higher when potassium predominates than when sodium predominates, suggesting that its uptake is linked with that of the cation. Again, the process appears to be closely geared to requirements in the sense that for saline-fed insects the rate of potassium uptake is greatly reduced at high ion concentrations (Fig. 5.3(b)), thus providing a mechanism by which a copious excretion of excess ions may be effected.

The uptake of substances other than water and inorganic ions from the rectal fluid has not been the subject of detailed study, but since a variety of solutes are known to enter the fluid of the Malpighian tubules, yet fail to make their appearance in the insects' excreta (see Chapter 6), it must be assumed that a process of resorption occurs in the rectum. Further studies will be needed to establish whether such absorption takes place by an active process against an electrochemical gradient, or whether it is a simple consequence of an increase in rectal concentration associated with the uptake of water.

One substance which is not resorbed by the rectal glands is uric acid, which enters the rectum as the soluble potassium salt in alkaline solution. In the rectum the pH of the urine is reduced from about 7.0 to between 4.0 and 6.0, possibly as the result of an active secretion of hydrogen ions by the rectal epithelium. This causes the formation of highly insoluble uric acid from its more soluble salt and, coupled with the resorption of water from the rectum, results in
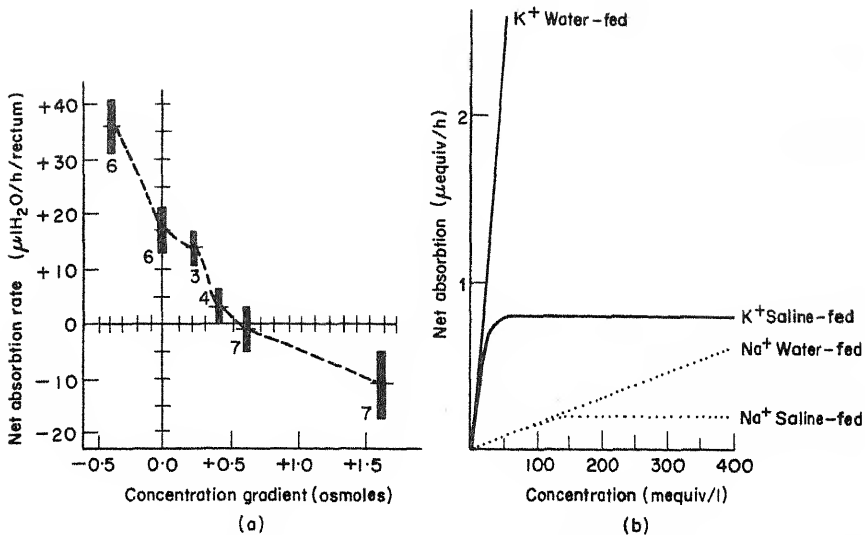
Fig. 5.3. Aspects of rectal absorption in the locust. (a) The relationship between the initial osmotic gradient and the rate of water movement across the rectal wall. A positive sign indicates absorption from the rectum (ordinate), or rectal fluid more concentrated than the haemolymph (abscissa); bars indicate the extent of standard deviations, and subscript numbers the number of observations (Phillips, 1964a). (b) The relationship between rectal fluid concentration and the net rate of absorption of potassium and sodium from the ligated rectum of the locust (redrawn from Phillips, 1964b).

a massive precipitation of uric acid. In view of the limitations set to the absorption of water by the osmotic gradient, the occurrence of uric acid as the main excretory product in insects is clearly of the greatest significance. The maximum osmotic effect which it can exert will be governed by its solubility in an acid medium, which is no more than 0.0004 osmoles/litre (6 mg/100 ml). Provided that other solutes are removed, water can therefore continue to be resorbed from the rectal contents without any increase in its osmotic concentration.

5. Regulatory Aspects

An attempt has been made to bring together the results described in previous sections in the form of a summary diagram, to serve as the basis for a discussion of regulatory aspects of excretion. Figure 5.4 shows the secretory cycling of potassium and water from haemolymph to Malpighian tubules and from rectum back to haemolymph, which forms the basis of urine formation and of osmoregulation. Also shown is the active secretion of uric acid, and the passive diffusion of other solutes, into the Malpighian tubule, followed by the
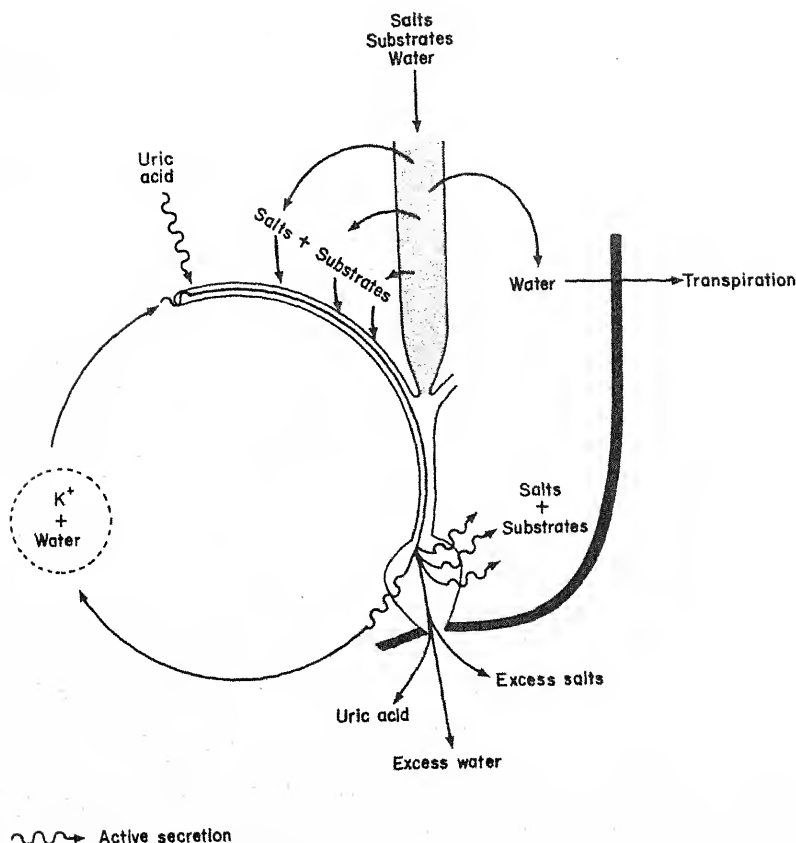
~~~→ Active secretion

Fig. 5.4. A diagrammatic summary of processes involved in the osmoregulation of insects; for further explanation see text.

elimination of uric acid from the rectum, and the recovery of salts and substrates, whether by active or isosmotic resorption. The different components of this system have been shown to be appropriately influenced by the water and salt requirements of the insect for it to serve as an effective basis of regulation. For instance, under conditions of desiccation, an increase in haemolymph osmotic pressure would cause a reduction in urine flow (see Fig. 5.2(iv)), and an increase in the absorption of water from the rectum (p. 70); the net effect would thus be to minimize excretory losses of water at a time when transpiratory losses are high. If the desiccating conditions involve an uptake of food with a high salt content, there would in addition be a decrease in the absorption of salts from the rectum (see Fig. 5.3(b)), and osmotic equilibrium would tend to be maintained in the face of declining water reserves by a correspondingly copious elimination of salts.

In insects under conditions tending to hydration, as in saturated atmospheres with access to fresh water but not to food, the danger would lie in the possibility of excessive dilution of body fluids. This would be counteracted by the increased urine flow and decreased rectal absorption of water associated with low haemolymph osmotic pressure; and coupled with an increase in the absorption of salts from the rectum, the result would be an elimination of water with limited loss of inorganic ions.

Some of these regulatory effects (e.g. increase in osmotic pressure leading to a decrease in rate of urine formation) may reasonably be attributed directly to changes in the magnitude of electrochemical gradients across the secretory epithelia. But the decrease in the absorption of salts, and increase in the absorption of water, which characterizes saline-fed animals must clearly be based on some indirect effect on the activity of the secretory cells, and it seems that effects of this kind may be mediated by blood-borne factors of the neuroendocrine system. One of the best documented examples of the hormonal control of excretion is furnished by the work of Maddrell on the blood-sucking bug *Rhodnius*, in which intense diuresis occurs soon after feeding. This coincides with the appearance in the haemolymph of a very active diuretic hormone, which appears to be released from a series of swollen nerve fibres associated with the abdominal nerves immediately behind the metathoracic ganglion.

While the mechanisms discussed above would provide a basis for the regulation of total osmotic pressure, the phenomenon of ionic regulation has still to be accounted for. This would involve a filtering off from the general haemolymph pool of ions which are taken in with the food, and would pose special problems where the proportionate composition of the food differs substantially from that of the haemolymph, as is often the case. The mechanisms by which such differential elimination of haemolymph ions is achieved have not yet been studied in sufficient detail to provide a firm basis of interpretation, but it seems that discrimination would occur at two sites:

(a) with an increase in the haemolymph concentration of a particular ion the rate at which it would diffuse into the Malpighian tubule, and hence the rate at which it would pass into the rectum, would increase; and

(b) the resorption rate of the ion from the rectum would be decreased, and become independent of rectal fluid concentration (ref. Fig. 5.3(b)) as a result of the increase in haemolymph concentration.

In other words, the rate at which the ion passes into the rectum increases, while the rate at which it is absorbed from the rectum decreases, so that the net result would be a selective elimination of the particular ion.

A great deal of effort has been devoted to the satisfactory elucidation of osmoregulatory mechanisms during recent years, but there has as yet been little work to determine the extent to which these mechanisms are effective in ensuring a regulation of ionic and osmotic concentration of body fluids in the

Fig. 5.5. Osmotic pressure of the haemolymph of the desert cockroach plotted against the insect's water content. The line represents the expected relationship in the absence of regulation (redrawn from Edney, 1966).

normal terrestrial insect. Changes in the haemolymph osmotic pressure of a desert cockroach during cycles of dehydration and rehydration are shown in Fig. 5.5, for comparison with the effects which would have been observed in the absence of regulation. The results show quite clearly that substantial changes in water content are reflected in relatively small changes in haemolymph osmotic pressure, indicating that regulatory processes are at work, and it is likely that some of the mechanisms discussed in this chapter may be involved; for instance an increase in the uptake of water, and a decrease in the resorption of salts, from the rectum during dehydration. But under the conditions of experiments of this type, with insects denied access to food, it is possible that the main factor involved in regulation may be an interchange of water and salts between haemolymph and tissue fluids. For a fuller elucidation of the mechanisms involved under natural conditions, and of their relative contribution to the total effect, determinations of haemolymph volume and of the water and salt content of the faeces would have to be included, and the experiment should preferably be carried out under conditions of controlled access to food and water, and under different conditions of desiccation stress.

NITROGENOUS WASTE PRODUCTS

The disposal of surplus nitrogen is a problem that confronts most animals, because the intake of nitrogenous material is usually greatly in excess of the demand. It is only during periods of rapid growth that the reverse is likely to be true, and even then it will usually be the availability of some particular component which is limiting (see Chapter 2), so that a substantial amount of nitrogenous waste will still require to be excreted.

The problem of nitrogen disposal is an acute one, because although nitrogen itself is not a toxic substance, the form in which it generally appears in metabolism is, for the primary nitrogenous end-product both of amino acid and of purine metabolism is ammonia (see Chapter 1). The precise basis of ammonia toxicity has not yet been established, but it appears to be largely independent of the changes in pH which would be associated with the formation of ammonium hydroxide in aqueous media.

Certain aspects of the formation and disposal of nitrogenous waste products have been dealt with in earlier chapters. The metabolic pathways involved in the detoxication of ammonia, by incorporation of the nitrogen in uric acid, have been described in Chapter 1; and the mechanism by which uric acid, as the primary nitrogenous end-product, accumulates in the rectum for ultimate disposal by defaecation has been discussed in Chapter 5. What remains to be dealt with here are the quantitative aspects of the problem, the proportion of different nitrogenous waste products excreted by different insects, and methods of disposal of such waste products other than by normal excretion.

1. Excretion of Waste Products

It has long been recognized that uric acid is the main vehicle of nitrogenous excretion in terrestrial insects, and the class as a whole has generally been regarded as a satisfactory example of the so-called "uricotelic" mode of life, with uricotelism seen as a specific adaptation to the terrestrial habitat. It is argued that most aquatic animals are in a position to permit a relatively free exchange of small and readily diffusible molecules, like ammonia, between the

internal and the external environment, either across the general body surface or across special respiratory epithelia. Under these circumstances they would be able to dispose of excess nitrogen by simple diffusion of ammonia across permeable membranes, at a rate which would be sufficient to prevent the build-up of ammonia in the body fluids to toxic levels. One of the primary requirements for the invasion of a terrestrial habitat was a drastic reduction in general permeability to water, which appears to have been accompanied by a corresponding reduction in the permeability to other molecules, like oxygen and ammonia. That it is the permeability of the integument which is of importance in this context, rather than simply the exchange of an aqueous for an aerial medium, is well illustrated by the terrestrial isopods, which have retained a permeable cuticle and continue to excrete the bulk of their nitrogenous waste as ammonia, by simple diffusion across the integument. In most other terrestrial groups, where ready diffusion of ammonia into the atmosphere is precluded, the need arose to develop some mechanism of detoxication, and this was met by the formation of urea in ureotelic groups and uric acid in uricotelic groups. Both of these substances, being non-volatile, would require an output of urine for their disposal, so that it is at this stage that the osmoregulatory system comes to take on a secondary role as an excretory system. Urea would serve as a satisfactory waste product in animals which had sufficiently large water reserves, or sufficiently ready access to water, to permit the loss of substantial quantities of water as hypotonic, or slightly hypertonic urine. In many terrestrial insects, however, access to water may be unreliable and infrequent, and because of their small size water reserves are minimal. For such insects an advantage would be gained by reducing losses of water by excretion, and this could not readily be done with urea as the main nitrogenous waste product, because of the high solubility of this substance. Withdrawal of water from the urine would be militated against by a progressive increase in the osmotic pressure exerted by such a soluble excretory product, and a low limit would be set to the hypertonicity of urine by the absorptive powers of the rectal epithelium (see Chapter 5). Under these circumstances the insolubility of uric acid would clearly give it a considerable advantage as an excretory product, because the osmotic pressure of a saturated solution of uric acid is well below the absorptive capacity of rectal epithelium, so that, in the absence of other soluble material, an all but complete withdrawal of water from the excretory product should be possible. The virtually dry excreta produced by many species of insect attests sufficiently to the importance of uric acid as a principal excretory product.

It should not be forgotten, however, that the insect pays a heavy price for the benefit which it derives in terms of water balance. The synthesis of a uric acid molecule involves the expenditure of substantial amounts of energy (see Chapter 1), and the four atoms of nitrogen which it contains are associated with five atoms of carbon, which might otherwise have featured to advantage in the

context of synthetic and degradation metabolism. The nature of the bargain struck is well illustrated in blood-sucking insects, such as the tsetse fly, where the type of food imposes a particularly high nitrogen load. It can be calculated that for every 100 mg dry wt of blood ingested, no less than 47 mg have to be excreted to ensure the disposal of surplus nitrogen; and losses of energy associated with the manipulation of the blood meal (digestion, absorption, uric acid synthesis, excretion etc.) reduce the net gain to the insect to something like 50 mg of respirable material. If the insect had been able to dispose of the nitrogen by simple diffusion as ammonia, the corresponding value would be in the region of 85 mg.

The necessity to produce uric acid thus constitutes a serious disadvantage in terms of metabolism, but one that is outweighed by its advantage in the context of water balance, and uric acid has in fact been shown to constitute the main nitrogenous end-product in terrestrial insects. Within the limits of this broad generalization a situation of considerable complexity has been shown to exist, whose details unfortunately remain obscure for lack of sufficiently extensive information. The aim of investigators in the field of excretory metabolism has seldom been the evaluation of total nitrogen balance; many have been concerned simply to demonstrate the presence or absence of a specific material in the excreta, and even where information is available concerning a range of nitrogenous materials, this has rarely been coupled with determinations of total nitrogen, so that unequivocal assessment of the precise proportion which a given material, such as uric acid, constitutes of the total nitrogen cannot be made. For purposes of a general comparison between the few species for which reasonably extensive information is available, the nitrogen content of each substance has been expressed as a percentage of the total nitrogen content of the substances assayed. These will usually include the most important, though the possibility

TABLE 6.1

The percentage distribution of nitrogen among different excretory products in adult terrestrial insects

| Order | Species | % of total nitrogen | | | | |
		Uric acid + primary degradation products	Amino acids	Urea	Ammonia	Ref.
Orthoptera	*Melanoplus bivittatus*	55	11	4	29	1
Heteroptera	*Dysdercus fasciatus*	61	15	12	–	2
	Rhodnius prolixus	97	trace	3	–	3 + 4
Diptera	*Aedes aegypti*	66	7	15	12	5
	Glossina morsitans	82	15	trace	–	6

1. Brown, 1937   4. Harrington, 1961
2. Berridge, 1965   5. Irrevere and Terzian, 1959
3. Wigglesworth, 1931   6. Bursell, 1964

cannot be excluded that unidentified substances, or substances not quantitatively determined, may exceed the listed materials in importance, and Table 6.1 is presented with this qualification.

a. Uric Acid and its Primary Degradation Products

The nitrogenous excretion of insects has been the subject of fairly intensive investigation during recent years; in most of the species investigated, which include representatives of most of the orders, the bulk of excretory nitrogen appears in the form of uric acid, and in some virtually all of the excretory nitrogen is in this form. Even in species where uric acid is not the predominant excretory product it usually accounts for a substantial proportion of excretory nitrogen; and it is absent from the excreta only in a very few cases, where the use of other excretory end-products may be seen as a special adaptation to a particular mode of feeding. In certain plant-sucking insects, such as the cottonstainer, for instance, the need to dispose of large quantities of inorganic ions from the diet appears to have involved a shift from uric acid to allantoin as the main excretory product, and uric acid is completely absent from the excreta.

Substantial quantities of the primary degradation products of uric acid (allantoin and allantoic acid (see Chapter 1)) occur in the excreta of insects from most of the orders. In some, allantoin may be the predominant material, with uric acid and allantoic acid present in variable proportions ranging from zero to 30% or more of the total excretory nitrogen. In others, especially among the Lepidoptera, allantoic acid predominates, sometimes with allantoin and sometimes with uric acid as co-dominant. It has not been possible to establish any convincing correlation between the quantitative importance of one or other of these substances and other aspects of biology or of taxonomy. A given pattern of distribution of nitrogen among the three substances may occur among members of primitive or advanced orders, among insects with widely different modes of feeding, and indifferently in early or late developmental stages. The predominance of a particular end-product cannot, therefore, be regarded as a species characteristic, but becomes descriptive simply of a particular moment in the life history of a particular insect, and for this reason there seems at present little point in distinguishing too rigidly between the different patterns of excretion. Effort should be directed, however, towards an elucidation of their biological significance; the fact that the enzymes concerned with the primary degradation of the purine ring, uricase and allantoinase, appear to be particularly active in extracts from the Malpighian tubules and midgut, both of which have been shown to be associated with the elimination of excretory products, suggests that degradation of the purine ring may in some way be involved with the transfer of material across the secretory epithelium. But until this and other possibilities have been explored, it would seem preferable to regard the occurrence of high proportions of allantoin and/or allantoic acid as minor

variations on a basic uricotelic theme, especially since these substances differ relatively little from uric acid in terms of the properties which are chiefly relevant in the context of excretory metabolism, namely nitrogen content and solubility. It would, on this view, be legitimate to retain the notion of terrestrial insects as predominantly uricotelic, defining a uricotelic animal as one that uses uric acid, allantoin or allantoic acid, or some mixture of the three, as the main excretory material.

b. Urea

Urea is present in the excreta of most insects which have been investigated, and it makes up a substantial proportion of total nitrogen in some (see Table 6.1), but the role of this material in nitrogenous excretion is poorly understood. Its presence cannot be accounted for on the basis of purine degradation, because no insect has been shown to possess the full complement of enzymes required to degrade uric acid to this stage. On the other hand, there is little evidence for the existence of an ornithine cycle in insects. The presence of arginase has been demonstrated in several species, catalysing the hydrolysis of arginine to urea and ornithine, and arginine itself is of importance as a phosphagen, a store of high energy phosphate, in insects. It is possible, therefore, that excretory urea may arise by the hydrolysis of arginine, but if so, the biological significance of the reaction remains to be elucidated.

c. Ammonia

Ammonia has been identified in the excreta of most insects in which it has been carefully looked for, and in some it may account for a substantial proportion of total nitrogen (see Table 6.1). Whether it should be regarded simply as a fraction of the total ammonia arising in the course of deaminations that fails to become detoxicated by incorporation in the uric acid molecule, or whether its occurrence in the excreta is of some positive significance in relation to excretory function, has not yet been determined.

d. Amino Acids

A variety of amino acids have been identified in the excreta of various insects, but the quantities involved are often so small that their occurrence could legitimately be regarded as reflecting a failure of resorption by the rectal epithelium (see Chapter 5) rather than as a normal part of the excretory process. In some insects, however, particularly among species which subsist on high protein diets, the quantities are substantial (see Table 6.1) and the distribution among different kinds of amino acids bears relation neither to the proportionate composition of the haemolymph, which would be reflected in that of the urine, nor to the proportionate composition of the diet, which might be reflected in that of digestive wastes, so that in these a selective elimination through the

excretory system would appear to be involved. In the tsetse fly the main amino acids in the excreta are arginine and histidine, the two that have the highest nitrogen content of all protein amino acids, and their elimination could perhaps be seen as a method by which the metabolic losses involved in the synthesis of uric acid are minimized during the first half of the hunger cycle, when water reserves are plentiful.

e. Miscellaneous Materials

Pteridines occur in high concentration in the excreta of certain insects, but little is known of their excretory metabolism. Xanthine and hypoxanthine, reduced derivatives of uric acid (see Chapter 1), have been identified in the excreta of a few insects, sometimes making up as much as 10% of the total nitrogen. In blood-sucking insects haematin, or haematin derivatives, are conspicuous components of the excretory material.

2. The Storage of Excretory Products

The deposition of excretory products in various tissues of the body seems to be a regular feature of many species of insect. It is particularly common among members of the Orthopteran family Blattidae, in which as much as 10% of the total dry weight of the body may be uric acid. In many insects the uric acid is stored in the fat body, and in view of the readiness with which it may be mobilized from such deposits, and of the possibility that it may serve as a source of nitrogen for synthetic purposes, use of the term storage excretion may not be altogether appropriate. In other cases, however, the sequestration of uric acid seems to be permanent, as in its deposition in the cuticle of certain species; or the material may be destined for eventual elimination, as in certain cockroaches where large quantities of uric acid are deposited in the accessory sex glands, from which they are poured over the spermathecae during copulation; here a true disposal of nitrogenous wastes would seem to be involved.

RESPIRATORY EXCHANGES

Perhaps the most striking feature of the organization of insects is the tracheal system. It represents in essence a system by whose means air is brought into the closest possible contact with sites of tissue respiration in all parts of the body. Air may be said to be piped to the respiring tissues, and their respiratory needs are thus taken care of in a very direct fashion. The insects are not unique in the development of such a mode of respiration, but it is in them that it reaches its finest expression, and the tracheal system of insects has attracted the attention of anatomists and physiologists alike, through the centuries.

The main function of the respiratory system is to enable an efficient exchange of the respiratory gases, oxygen and carbon dioxide, which feature respectively as an indispensable input and as an unavoidable output of the metabolic system. The fulfilment of this function is linked to the provision of a permeable membrane across which the respiratory exchange can take place, and a conflict of requirements arises in this connection. For it seems that, in most natural membranes, permeability to oxygen goes hand in hand with permeability to water, and a membrane across which oxygen can exchange freely is also one across which water can be readily lost to a dry atmosphere. This is a problem which faces all terrestrial animals, and in all it leads to a compromise in the sense that a balance is struck between the need to meet the demands of respiration on the one hand, and the necessity to limit respiratory exchange in the interests of water balance on the other. So closely interlinked are the processes of oxygen uptake and water loss, of respiration and transpiration, that they cannot sensibly be treated in isolation from one another. Unless the respiratory system is seen against the background of this compromise, many of its properties cannot be interpreted satisfactorily. What must be discussed in this chapter is, therefore, not only the exchanges of oxygen and of carbon dioxide, but also the losses of water which are unavoidably sustained in the process of respiration. Thus, while it is the respiratory system which is under consideration, discussion will not be limited to the exchange of respiratory gases.

Unfortunately the area of overlap between respiration and water balance is one that has been much neglected by experimental physiologists, who tend to

fall into distinct categories depending on whether they are interested primarily in respiration, or primarily in water balance. As a result, a great deal of information may be available concerning details of respiratory function in one insect, and of the mechanisms by which transpiration is regulated in another, but there is no single instance of a concerted experimental attack on both aspects of the question in a single species. In order to present a coherent account, some play has unavoidably had to be given to speculation, in order to marry the results obtained from two quite distinct disciplines of physiological investigation.

1. The Structure of the Respiratory System

Before entering on a discussion of respiratory physiology, it will be necessary to give some account of the structure of the respiratory system, which is one of considerable complexity. Its main features are illustrated diagrammatically in Fig. 7.1, which indicates how oxygen is supplied to the flight musculature of an insect. The tracheal system opens to the thoracic surface at the spiracle, which is often set in a cuticular depression, and whose opening is usually guarded by a filter of cuticular bristles, serving to prevent entry into the tracheal system of

Fig. 7.1. Schematic illustration of the tracheal system of an insect. 1, spiracle; 2, filter; 3, atrium; 4, spiracular valve; 5, tracheal manifold; 6, primary trachea; 7, secondary trachea; 8, tertiary trachea; 9, tracheole; 10, tracheal end cell; 11, sarcolemma; 12, mitochondrion; 13, tracheal epithelium; 14, taenidium.

particulate material. An atrial cavity may be interposed between the filter and the spiracular valve; the valve itself takes a diversity of forms in different species of insect, but essentially it is a relatively simple system of cuticular levers operated by one or more bands of muscle, whose contractions cause the levers to bear on the tracheal tubes in such a way as to reduce their diameter to the point of total occlusion. Beyond the valve the trachea usually divides into a number of main branches, some of which establish communication with other parts of the tracheal system as longitudinal or transverse connectives. Further branching gives rise to secondary and tertiary tracheae of progressively smaller diameter, and these in turn branch to form a series of fine tracheoles, which represent the final ramifications of the branched system, usually tapering from a diameter of $1.0\ \mu$ to end blindly at a diameter of $0.1\text{-}0.2\ \mu$. Each tracheole lies within a single palmate cell, the so-called tracheal end-cell, whose nucleus is usually situated near the origin of the tracheole. The tracheolar branches are associated with a sheath, representing projections fom the surface of the end-cell.

In flight muscles the tracheoles often penetrate into the muscle cells themselves, carrying with them not only the sheath of the end-cell but also the invaginated membrane of the muscle cell. They branch within the muscle fibre to invest each intracellular myofibril, in the closest proximity to the longitudinal arrays of mitochondria. In other organs tracheoles do not usually penetrate the individual cells, and they may do no more than form a general surface investment of the tissue.

Developmentally the tracheal system represents an invagination of the epidermis, and each trachea is surrounded by a sheath of epithelial cells supported by a basement membrane, and lined with a substantial cuticular membrane (see Fig. 7.1). The cuticular intima is thrown into folds which run a spiral course to form the so-called taenidia, serving to strengthen the tubes against collapse. The tracheal lining is composed of layers similar to those of the surface cuticle, but in the tracheole the intima becomes extremely delicate, and its composition has not yet been determined.

The finest tracheolar branches are often partially filled with fluid, and the point to which the liquid column extends is thought to depend on a balance between the capillary force, tending to draw liquid out along the tube, and the colloid osmotic pressure exerted by the cytoplasm of the end-cell. It has been shown that an increase in haemolymph osmotic pressure, as during muscular exercise, particularly under conditions of low oxygen tension, is associated with absorption of fluid from the tracheoles, leading to an extension of air into the finer branches, as illustrated in Fig. 7.2. This would obviously serve to facilitate respiratory exchange between the tracheole and the site of respiration.

The distribution of the tracheal system varies enormously from species to species, and from stage to stage in the life history. In many insects each body segment bears a spiracle, and different segments are linked by lateral longitudinal

trunks. There is, however, a general tendency towards reduction in the number of functional spiracles, and in some insects only the posterior pair of spiracles remains open, to serve the needs of the whole animal by way of the longitudinal trunks. Special thin-walled and collapsible dilatations, or air sacs, are a common feature of the tracheal system in many insects, occurring either as blind diverticula, or along the course of major tracheal routes. The precise distribution

Fig. 7.2. The extent of gas in one group of tracheoles in the abdomen of a flea at rest and exposed to different concentrations of oxygen (Wigglesworth, 1965).

of the finer tracheae at any one stage in the life history may depend to a considerable extent on local demands during the previous stage, and if insects are maintained at low oxygen tensions during development, the tracheal investment of particular organs may be increased. Within a given developmental stage there is a tendency for tracheoles to migrate towards regions of low oxygen tension. In other words, while the gross pattern of tracheal distribution is determined genetically, there is scope for fine adjustment in relation to local needs, thus ensuring that the supply of oxygen to various tissues is related to the demand for oxygen by those tissues.

Attempts have been made to measure the total volume of the tracheal system of a number of different insects by a variety of methods, and values ranging from 5% to 50% of total body volume have been reported. The total number of tracheoles in a silkworm larva has been estimated as 1.5×10^6, and in view of the high surface to volume ratio which would characterize tubes of such fine dimension, there can be little doubt that they constitute the main site of respiratory exchange.

2. The Physiology of Respiration

There are two different ways in which respiratory gases can be transported in the tracheal system of insects—by bulk flow of air, or by diffusion in still air. Diffusion may be free in the sense that it is limited only by the fixed dimensions of the tracheal system, or it may be restricted by closure of the spiracular valves. For purposes of discussion it will be convenient to consider the physiology of respiration under the three corresponding headings of (a) diffusion in the open system, (b) diffusion in the regulated system and (c) ventilation.

a. Diffusion in the Open System

(i) Oxygen. The question whether the oxygen requirements of an insect can be met by diffusion of oxygen through the complex ramifications of the tracheal system is one which has occupied the attention of physiologists for several decades. Recent calculations have served to confirm the early estimates of Krogh, who in 1920 showed that diffusion is a major factor in the transport of oxygen through the tracheal system; and that only a small gradient of oxygen tension is required to account for the transfer of oxygen from the mouth of the tracheal tree to the site of tissue respiration, at a rate sufficient to meet the demands of oxygen consumption.

It is important to note that the rate at which oxygen diffuses in air is enormously greater than the rate at which it diffuses in water. The permeability constants are 11.0 and 0.00003 ml, $min^{-1}.,cm^{-2}.,atm^{-1}.,cm^{-1}$ respectively, implying that if a given gradient of oxygen tension suffices for the transport of oxygen along a 3-cm long tracheal tube, that same gradient would be adequate for a diffusion distance of no more than $0.1\,\mu$ in the aqueous medium of the cellular fluid. This emphasizes the need to bring the tracheolar supply into extremely close proximity to the site of tissue respiration, and Weis Fogh (1961) has calculated that during flight in the dragonfly, with the partial pressure of oxygen in the tracheoles at 142.5 mmHg, which is close to the 150 mmHg of the atmosphere, the maximum distance for tissue diffusion is about $10\,\mu$. In other words, unless the mitochondria are within a distance of $10\,\mu$ from the nearest tracheole, the rate at which oxygen can be supplied to them would be insufficient to meet the demand. In actual fact, the tracheoles of active flight muscle are usually no more than $3\,\mu$ apart, so that the architecture of the system provides a substantial safety factor.

Such measurements as have been made of the tension of oxygen in tissue fluids and in the tracheal system of insects confirm that the drop in tension in the aerial phase is slight, probably of the order of 2% during rest, and 20-30% during flight, when the rate of transport has to be increased to meet the greater demand.

An attempt has been made to provide a diagrammatic summary of the situation in Fig. 7.3(a), where curve (i) represents the oxygen tension at

Fig. 7.3. Diagram to illustrate the disposition of tension gradients for oxygen, carbon dioxide and water in the unventilated tracheal system of an insect. (a) Spiracles open. (b) Spiracles closed. Since the cross-sectional area of the tracheal tree does not change substantially along its length, and since the bulk of respiratory exchange would take place through the relatively enormous surface area of the tracheolar branches, the system has been represented as a single unbranched tube extending between the atmosphere on the left and the site of tissue respiration, as represented by a mitochondrion, on the right. The linear scale has been greatly distorted, as indicated at the bottom of the figure, to allow graphical representation of the whole system. It is assumed that the respiratory gradients shown for the insect at rest are adequate to satisfy the needs of steady-state respiration at the rate of $x \, \mu lO_2/min$, and that respiratory rate during activity is 5 x; for further explanation see text.

different points along the diffusion path from the atmosphere on the left to the mitochondrion on the right, in an insect at rest. A very shallow gradient suffices for transport in the aerial phase, a much steeper one is required for transfer in the aqueous phase from the end of the tracheole into the cell and across to the mitochondrion, and it is at this point that most of the drop in tension occurs. Even so, the oxygen tension at the mitochondrial surface is represented as being relatively high, and reduced by no more than 20% from atmospheric.

During activity, or during exposure to high temperatures, when the rate at which oxygen is consumed would be substantially increased, the gradients would need to be correspondingly steeper to promote faster transport, as indicated in curve (ii) of Fig. 7.3(a). This can only be achieved by dropping the tension at the mitochondrial surface to a much lower level, one, however, that would still be adequate for maximal mitochondrial activity. In this case, again, the bulk of the gradient has to be exerted across the aqueous pathway.

(ii) Carbon Dioxide. The carbon dioxide which is produced at the site of tissue respiration will combine with water to give carbonic acid, and this, in turn, will dissociate to give the bicarbonate ion. The combination of carbon dioxide with water is a relatively slow reaction, and in the blood of vertebrates it is accelerated by an enzyme, carbonic anhydrase, present in the red blood corpuscles. This enzyme has not been demonstrated in the haemolymph of terrestrial insects, but it has been shown to be active in the tissue fluid of several species. This would be in accord with the fact that insect haemolymph plays little part in the transport of respiratory gases, and that the bulk of exchange occurs directly between tissue and tracheoles.

The bicarbonate content of insect haemolymph, and of the tissue fluids with which it is in equilibrium, is variable, and the relation between carbon dioxide tension and bicarbonate concentration (the CO_2-capacity) in insect haemolymph also differs widely between species. For present purposes a value of 5 m.eq/litre of bicarbonate in equilibrium with an atmosphere containing 2% carbon dioxide will be assumed, giving a tracheal carbon dioxide tension of about 15 mmHg. With the carbon dioxide content of the atmosphere at less than 0.1%, the gradient available for promoting the diffusion of carbon dioxide from tissue to environment would then be about 15 mmHg. This is very much less than that which promotes the transfer of oxygen in the reverse direction. The permeability coefficient for carbon dioxide in an aqueous medium, however, is about 36 times greater than that for oxygen, so that a much shallower gradient between mitochondrial surface and tracheole wall would suffice, while the gradient in the aerial phase would need to be very little greater, since the diffusion coefficient for carbon dioxide in air is only marginally smaller than that for oxygen. Conditions would thus be adequate for the disposal of carbon dioxide along a gradient system such as that illustrated in Fig. 7.3(a), curve (iv).

During activity carbon dioxide would be produced at a greater rate, and it

would have to be disposed of correspondingly faster over a correspondingly steeper gradient system. The only way in which this could be achieved would be by raising the tension of carbon dioxide at the tissue level, and thus it has been represented in curve (iii) of Fig. 7.3(a); it should be mentioned that experimental evidence for an increase in carbon dioxide tension during activity is lacking.

Since carbon dioxide can diffuse more rapidly than oxygen through water and through lipids, the proportion of carbon dioxide which exchanges through the general body surface, rather than through the tracheal system, would be proportionately greater. In experiments with insects whose spiracles have been blocked, substantial diffusion of carbon dioxide takes place through the general body surface, but it is likely that this would be associated with abnormally high internal tensions of carbon dioxide. Under normal conditions the fraction that exchanges through the body surface is probably small, and it may be ignored in the present context.

(iii) Water Vapour. No careful theoretical approach has yet been made to the problem of the disposition of gradients of vapour pressure within the tracheal system of insects, nor are experimental data available that could serve as a basis for a theoretical model. The account which follows will therefore be superficial and speculative, and will have to be regarded as no more than a first rough approximation.

The total gradient available to promote the diffusion of water vapour from the insect to the environment is set, on the one hand, by the saturated vapour pressure of air in equilibrium with the tissue fluids, and on the other, by the water vapour pressure of the atmosphere. With an insect exposed to dry air at a temperature of $25°$ it would amount to about 25 mmHg, with the gradient extended between the tissue fluids in contact with the tracheolar wall and the mouth of the tracheal tree. The tracheoles appear to be lined by a layer of cuticulin (see Chapter 1) constituted by a tanned lipoprotein complex. No information is available concerning the permeability of this layer, but it seems reasonable to suppose that it would offer substantial resistance to the diffusion of water, and so it has been represented in curve (v) of Fig. 7.3(a); approximately half of the total gradient is represented as acting across the tracheolar membrane. In this case the disposition of gradients would, of course, be the same in resting as in active insects, except in so far as the rise in temperature associated with activity would slightly increase the saturated vapour pressure.

b. Diffusion in the Regulated System

(i) Water Vapour. The rate at which water is lost from the tracheal system under the conditions illustrated in Fig. 7.3(a), that is, with spiracles open, has been determined for a number of insects, and has been demonstrated to be

substantial. This in itself indicates that a considerable gradient of vapour pressure is acting across a relatively permeable respiratory membrane, so that the satisfaction of respiratory requirements may be seen as posing a threat to water balance. The way in which this threat has been countered in most classes of insects is by the development of a mechanism for closing the spiracle, in other words, by the interposition of resistance to diffusion at one point in the diffusion pathway, as illustrated in Fig. 7.3(b). Since diffusion would be greatly impeded by occlusion of the tracheal lumen, it is clear that a great part of the available gradient would under these circumstances have to be exerted across the region of resistance, as illustrated in curve (i) of Fig. 7.3(b). As far as water vapour is concerned, the result is that a relatively small proportion of the total gradient is now exerted across the respiratory membrane, and the rate at which water is lost through that membrane, and hence from the system as a whole, will be correspondingly reduced. Quantitative aspects of this effect are illustrated in Fig. 7.4, which shows the water loss of tsetse flies at different relative humidities. In air containing 15% carbon dioxide the spiracles are kept permanently open, the rate of water loss is high and directly proportional to relative humidity (Fig. 7.4, curve i), as it would be in a simple physical system. The rectilinear relation is maintained when the spiracles are artificially blocked, but under these circumstances the rate of water loss is greatly reduced (Fig. 7.4, curve iii), representing transpiration through the general body surface. With the

Fig. 7.4. The rate of water loss of tsetse flies at different relative humidities. (i) In air containing 15% carbon dioxide. (ii) In air. (iii) In air with spiracles blocked. The degree of effective closure of the spiracles is indicated at four different levels of humidity; for further explanation see text (schematized from Bursell, 1957).

normal insect in air free of carbon dioxide, the rate of water loss is intermediate between these two extremes, and its relation to relative humidity shows a marked departure from rectilinearity, with water loss strongly reduced at low humidities (Fig. 7.4, curve ii). The difference between curves (i) and (ii) at any one point, expressed as a proportion of the difference between curves (i) and (iii) at that same point, gives a measure of the degree to which the spiracles are closed, and values have been included at representative points of the curve. They show that in dry air the effective mean spiracular aperture is reduced by 90%, while in humid air the extent of occlusion is very much less. Whether these values indicate that the spiracles are held at the corresponding degree of closure, or whether they indicate that the spiracles are kept closed for the corresponding proportion of time, has not yet been determined; the subject will be discussed further in Chapter 10, where neurophysiological aspects of spiracular regulation will be considered.

(ii) Carbon Dioxide. The distribution of gradients for carbon dioxide will be affected in a way similar to those for water as a result of spiracular occlusion, and to ensure the disposal of carbon dioxide at the rate at which it is produced by the resting insect, the carbon dioxide tension at the site of respiration would need to be raised to more than twice its former value, as indicated by curve (ii) of Fig. 7.3(b).

(iii) Oxygen. With oxygen, too, a considerable proportion of the available gradient will have to be extended across the spiracular valve, so that, to maintain a transfer at a rate equal to the rate of consumption, the tissue tension would have to be dropped from about 120 mmHg to about 90 mmHg (see curve (iii) of Fig. 7.3(b)). The diagram thus illustrates in more specific terms the existence of a conflict between the requirements of water balance and of respiration, in so far as it shows how conditions which favour the supply of oxygen (open spiracles) favour also the loss of water, while conditions that reduce losses of water (closed spiracles) will jeopardize respiratory requirements to some degree.

It is clear from Fig. 7.3(b) that under the hypothetical conditions illustrated, the tension of oxygen at the site of respiration is still well above that which is required for maximal mitochondrial activity. It is not until the active animal is considered that the conflict finds full expression. On the assumption that oxygen consumption increases five-fold, the gradient across the spiracle would need to be five times as steep in order to supply oxygen at the requisite rate, and so would the gradient along the main tracheal pathways. Gradients of this magnitude are illustrated in curve (iv) of Fig. 7.3(b), which may be said to run out of oxygen tension long before the end of the pathway is reached, indicating that steady-state respiration at this rate would be impossible. The closure of spiracles would obviously militate against even a moderate rise in metabolic rate, to say nothing of the 50- to 100-fold increases which may characterize insects in flight. It is in fact a matter of common observation that bursts of struggling in

restrained insects are usually associated with momentary opening of the spiracular valves, while the onset of flight activity is accompanied by sustained opening. This suggests that during flight the requirements of respiratory exchange may take precedence over requirements of water balance; the spiracles are opened wide to permit access of oxygen, while water is allowed to be freely lost from the respiratory membranes. It is possible, however, that the conflict may be more finely balanced than that, even during flight. The spiracles might be opened no more than would satisfy the requirement for oxygen, so that although the rate of water loss is still high, it may not be completely unregulated. Very little work has been done on the water loss of flying insects, and such a possibility is by no means excluded by available evidence.

c. "Discontinuous Respiration"

The phenomenon of spiracular regulation was brought forcefully to the attention of respiratory physiologists during the 1950s, when anomalous results were obtained by a number of workers, who were investigating the respiratory exchange of quiescent or inactive insects. It was observed that, while the oxygen consumption proceeded continuously at a steady rate, the release of carbon dioxide occurred in intermittent bursts, as illustrated in Fig. 7.5(b). This rather puzzling phenomenon became the object of a series of intensive investigations by American workers, who used the diapausing pupae of certain large moths as convenient experimental material, and a satisfactory interpretation of the phenomenon of "discontinuous respiration" has been proposed on the basis of this work.

It was shown, in the first place, that the cyclical release of carbon dioxide could be abolished by intubation of one or more of the spiracles, indicating that the spiracular valves were implicated. This was confirmed by observations of the exposed spiracular valves during the burst cycle. Spiracles were kept firmly closed immediately following a carbon dioxide burst; a period then followed during which slight fluttering of the valves could be observed (see Fig. 7.5(a)), until eventually the spiracles would open wide to complete the cycle with the release of another burst of carbon dioxide.

Measurements of the intratracheal pressure and of the tension of oxygen and carbon dioxide showed that, after a burst, the oxygen tension drops steeply from atmospheric levels down to about 25 mmHg, at which level it is maintained until the next burst (see Fig. 7.5(c)). The intratracheal pressure drops slightly during the interburst period, while the tension of carbon dioxide rises slowly from about 25 mmHg at the end of a burst to 50 mmHg at the end of the interburst, falling sharply as soon as the spiracles open.

Results of this kind have served as the basis for an hypothesis of "discontinuous respiration" put forward by Buck (1958), who envisages the following sequence of events. At the end of a carbon dioxide burst the tracheal

Fig. 7.5. Respiratory exchange in the diapausing pupae of *Hyalophora*. (a) The degree of closure of spiracular valves at different points in the cycle of discontinuous respiration. (b) The release of carbon dioxide and the consumption of oxygen during discontinuous respiration. (c) The tensions of carbon dioxide and of oxygen in the tracheal system during discontinuous respiration. Based on Schneiderman and Williams (1953), Schneiderman (1960) and Levy and Schneiderman (1958).

system has been flushed with air, so that oxygen concentration is high and carbon dioxide concentration relatively low. Consumption of oxygen by the respiring tissues leads to a fall in oxygen tension, the supply of oxygen by diffusion from the atmosphere being at this stage prevented by the closed spiracular valve. The carbon dioxide which is produced during respiration is to a large extent held in solution as bicarbonate; since it does not appear as a gas, to balance the disappearance of oxygen, the net result is a fall in pressure within the tracheal system. As the amount of bicarbonate, and to a smaller extent of the gaseous carbon dioxide with which it is in equilibrium, builds up, the spiracles open minutely and intermittently during the period of "fluttering". Since the intratracheal pressure is less than atmospheric, there will be a bulk flow of air, containing 21% oxygen, into the tracheal system. As the oxygen continues to be used, the low intratracheal pressure will be maintained, ensuring a sustained in-flow of air, and a continuous replenishment of oxygen. With the progressive utilization of oxygen, the intratracheal gas becomes progressively enriched with nitrogen, and carbon dioxide tensions will also increase steadily until a critical point is reached, when a new burst is initiated and the spiracles open wide. At this stage there would be rapid equilibration between the

atmosphere and the tracheal system, oxygen diffusing in and nitrogen and carbon dioxide out along their respective gradients of tension. The carbon dioxide held as bicarbonate would be released as a result of the drop in intratracheal carbon dioxide tension. At the end of the period of equilibration, the spiracles close and the whole cycle is repeated.

It is a measure of the degree of specialization within the field of insect physiology, to which reference has already been made, that the phenomenon of "discontinuous respiration" has been considered and interpreted in all but complete isolation from its *raison d'être*, which can hardly be other than water balance. It is difficult to conceive what respiratory benefit could arise from the use of a system that involves a progressive fall in tracheal oxygen tension and a progressive rise* in carbon dioxide tension, and there appears to be general agreement that, as with other aspects of spiracular regulation, the system must find its ultimate interpretation in the context of water balance, but as yet there has been no experimental attack on this aspect of the problem. The theoretical treatment of Buck (1958) has indicated that the outward diffusion of water vapour would be impeded by the bulk inflow of air during the interburst period, so that the system is likely to be effective in the conservation of water. But there has as yet been no attempt to determine experimentally what the quantitative implications of discontinuous respiration are in terms of water balance; indeed, the only experimental evidence that it is related to water balance at all is circumstantial, namely that pupae maintained at high relative humidities release carbon dioxide continuously.

d. Ventilation of the Tracheal System

The diffusion of respiratory gases between the atmosphere and the site of respiration is adequate to account for the respiratory exchange of many insects both during rest and in flight. In some, however, and particularly among the larger species, the diffusion path appears to be too long for respiratory requirements to be wholly satisfied in this way even in the resting insect, and in most, the process of diffusion becomes inadequate during the periods of intense metabolic activity which are associated with flight. In these a process of tracheal ventilation is superimposed on diffusion processes, oxygen within the tracheal system being replenished, and carbon dioxide flushed, by bulk flow of air in the larger tracheal branches. Diffusion is still the process by which respiratory gases are exchanged between the primary tracheae and the site of tissue respiration, and the main function of ventilation is simply to shorten the path of diffusion.

(i) Ventilation in the Resting Insect. Tracheal ventilation in the resting locust has been the object of a series of detailed investigations by Miller, the results of which form the basis of the following account. Four types of ventilatory movement have been distinguished, one involving the raising and lowering of the floor of the abdomen, one involving a telescoping of abdominal segments, and

two based on protraction and retraction of the head and of the prothorax respectively. The last three should be considered essentially as auxiliary mechanisms, which come into play only for short periods when the demands of respiratory exchange are intense.

The pressure exerted by muscular movements of the type described on the sprung walls of the larger tracheae cause alternate compression and expansion, leading to corresponding displacements of the air that they contain. In the resting insect about 86% of the total ventilation is associated with respiratory movements of the abdomen, with thoracic and cephalic components contributing no more than 11% and 3% respectively. Only about 5% of the total volume of the tracheal system is exchanged with each pumping stroke under normal conditions, but during hyperventilation the value may rise to 20%, and in other species as much as 70% of the total volume can be ventilated.

The frequency and amplitude of abdominal ventilation can be recorded very simply on a smoked drum by attaching a light lever to one of the abdominal sternites of a locust, restrained ventral surface uppermost by strips of plasticene. Examples of such records are shown in Fig. 7.6, where traces (a) and (b) show that the intensity of ventilation is greatly increased when the cephalic or the thoracic nerve centres are exposed to increased concentrations of carbon dioxide. Abdominal nerve centres are capable of initiating respiratory movements in their own segments, but these autonomous movements are of lower frequency and amplitude, and are insensitive to carbon dioxide. It appears that under normal conditions their rhythm is subject to the overriding influence of carbon dioxide sensitive pacemakers situated in higher centres.

The intensity of ventilation is also affected by oxygen lack, but at a much lower level of sensitivity; it is necessary to drop oxygen tensions to about half of their normal value to produce the same degree of hyperventilation as is produced by 1% carbon dioxide.

Earlier work had shown that the activity of spiracular valves was closely synchronized with ventilatory movements, in such a way as to produce a directed stream of air through the main tracheal trunks. This was confirmed by a detailed study of the behaviour of each of the 10 spiracles, and the results have been summarized in Fig. 7.6 (c) and (d). In the resting locust most of the

Fig. 7.6. Aspects of tracheal ventilation in the locust. (a) Tracing of kymograph records of the abdominal ventilation in the locust; the arrow marks the time at which air containing 6% carbon dioxide was injected into the cephalic tracheal system. (b) As for (a); the arrow marks the time at which the metathoracic ganglion was perfused with saline equilibrated with 5% carbon dioxide. (c) Schematic representation of the air flow resulting from ventilation during rest. Spiracles are numbered from 1 to 10, and the direction of flow is indicated by arrows. a.s., pterothoracic air sac. (d) Schematized representation of the closure of spiracular valves in relation to the phases of abdominal ventilation. I, inspiration; E, expiration. (e) Tracings of kymograph records of the abdominal ventilation in resting locust starved for 27 hr in dry air. (f) as for (e); after 27 hr starvation at high relative humidity. (a)-(d) from Miller, 1960; (e)-(f) from Loveridge, 1968.

(a)

(b)

1 Min

(c)

(d)

Spiracles ①+②
Open
Closed

Spiracles ③-⑨
Open
Closed

Spiracle ⑩
Open
Closed

Ventilation

I E I E I E I E I E I E

(e)

(f)

spiracles remain closed during all phases of the cycle of ventilation. Spiracles 1 and 2 open during early parts of the inspiratory phase, at which time all other spiracles are closed, so that air is drawn in through the first two spiracles. During expiration spiracles 1 and 2 close, and spiracle 10 opens, allowing air to escape through the posterior spiracle, and giving a flow through the main tracheal trunks as illustrated in Fig. 7.6(c). Under conditions of greater oxygen demand, other abdominal spiracles may be brought into use for expiration, in sequence from the posterior end.

It has been demonstrated recently that the intensity of ventilation in the locust is regulated in relation to the demands of water balance. During exposure to desiccating conditions there is a progressive reduction in the amplitude and frequency of abdominal ventilation (see Fig. 7.6 (e) and (f)). This is undoubtedly another reflection of the conflict that exists between respiratory requirements and the need to limit transpiratory losses of water. By reducing the rate of flow of air through the primary branches of the tracheal system the vapour pressure gradient between the mouth of secondary tracheae and the tracheolar membranes is reduced, thus decreasing the rate of water loss across the respiratory surface. By the same token, the gradient of oxygen tension would be decreased, so that the oxygen tension at the site of tissue respiration would need to be dropped in order to ensure a supply of oxygen commensurate with the demands of respiration; and similarly, the carbon dioxide tension would need to be increased in order to maintain the requisite gradient for disposal at the requisite rate.

Details of the ventilatory pattern differ substantially from species to species; in some the flow may be predominantly tidal, and directional air streams may be weakly developed; in others the air may enter posterior spiracles during inspiration and leave through thoracic spiracles. Whatever the precise pattern, ventilation is invariably confined to the larger tracheal tubes, and the transfer of gases in the finer branches of the tracheal tree must always take place by diffusion.

(ii) Ventilation During Flight. To meet the tremendous increase in the demand for oxygen during flight there is a marked change in the pattern of ventilation. In the locust this is reflected particularly in the behaviour of spiracle 2 and the bringing into operation of spiracle 3. At the onset of flight both these spiracles open fully, and in early phases of flight they remain open during all phases of abdominal ventilation, the frequency and amplitude of which shows a pronounced increase. The opening and closing of spiracle 1 remains in phase with abdominal ventilation, the only difference being that during early phases of flight it tends to open more fully than it does during rest. In addition, all of the abdominal spiracles take on the pattern of activity of spiracle 10 during rest, serving, that is, as additional points of exit for the air stream entering the anterior spiracles. During later phases of flight there is a tendency for spiracles 2

and 3 to close during abdominal inspiration, so that the contribution of spiracle 1 to the directional flow of air through the cephalic system and backwards to the abdomen will tend to increase. The over-all effect of the changed pattern of ventilation will thus be to increase the flow of air which characterizes the resting insect, and to superimpose on this a tidal flow to the flight musculature of the pterothorax, in and out of the thoracic spiracles.

Pterothoracic ventilation in the flying locust has been the object of detailed study by Weis Fogh (1961), who showed that while abdominal ventilation would be quite inadequate to provide for recorded consumption during flight, locusts with the abdomen completely removed were able to fly without sign of respiratory distress. It seems that not only is the pterothoracic tracheal system in some degree anatomically isolated from the rest of the tracheal system (see Fig. 7.6(c)), but it is capable of acting as an autonomous unit of ventilation. The tidal flow is brought about in part by small amplitude movements of the pterothoracic walls and not associated with the wingstroke, and in part by contraction of the wing muscles themselves, which ventilate the proximal parts of the secondary tracheae. The volume change during the wingstroke is quite small, but at 1040 strokes per min it delivers 750 litres of air per kg of flight muscle per hr, which is well above the level of requirement for the metabolic machinery, at about 600 litres/kg/hr.

3. Conclusion

It has been indicated that insects have solved the problem of respiratory exchange in a direct and very simple way, by bringing air rich in oxygen and poor in carbon dioxide into the closest juxtaposition to the site of respiration. But while the basic principle of tracheal respiration is simple, the mechanism by which it is achieved has in most insects become greatly elaborated in order to meet the demands of water conservation. This has been achieved by the development of spiracular valves which ensure, in one way or another, that the water loss associated with respiratory exchange is reduced to a minimum. The development of mechanisms for closing the spiracles has in turn opened the way to the production of a directional flow of air through the main tubes of the tracheal system, so that respiratory demands can, where necessary, be met in part by bulk flow rather than solely by diffusion.

SECTION II

Neuromuscular Physiology

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INTRODUCTION

In Section I the satisfaction of metabolic requirements has been considered in terms of somatic physiology, the processes of which ensure the requisite input of food materials and oxygen, the elimination of waste products and the maintenance of conditions appropriate to the proper functioning of the metabolic machinery. In that section the insect was considered largely as a self-contained entity, and the satisfaction of different metabolic requirements was discussed with reference to the corresponding organ systems. But an insect should obviously not be regarded simply as the sum total of a number of autonomous systems, since the continued existence of the whole depends very much on an appropriate co-ordination between the activities of its different parts. Nor is it legitimate to consider any metabolic system as self-contained and isolated from the environment which sustains it. The satisfaction of the requirement for food, for instance, involves a particularly complex interaction between the insect and its environment. At this stage attention must therefore be turned to the mechanisms by which co-ordination between the activities of different organ systems is achieved, and to the means by which the insect is enabled to respond appropriately to features of the environment which are of importance in relation to its continued existence; and it is at this point, therefore, that the properties and functions of the neuromuscular system of insects must be examined. The same approach will be followed as before, in that attention will be focussed primarily on those features of the neuromuscular physiology of insects which appear to set them apart from the generality of animals. The conduction and transmission of the nerve impulse, the contraction of muscle and the initiation of afferent input at the level of the sense organ will be considered first, as what may be termed the unit processes of nervous function. After that the integrative aspects of nervous function will be discussed, firstly in terms of the activity of component units and their interaction in isolated systems, and secondly as it is manifested in the complex behaviour of the insect as a whole, seen against the background of its neurophysiological basis. Such an approach may seem ill-advised at the present time, since our knowledge of neurophysiology is by no means adequate to provide a firm foundation for the interpretation of behaviour, nor is it likely that insects will prove particularly favourable material for an eventual bridging of the gap between the two disciplines of investigation. It has, nevertheless, been adopted

in preference to a purely empirical approach on the grounds that, though it cannot hope to provide a convincing interpretation of behaviour, yet it may give some indication of the general terms in which such an interpretation must ultimately be sought.

NERVES AND MUSCLES

1. Conduction of the Nerve Impulse

The nature of the nerve impulse and the mechanism of its conduction along the nerve fibre appear to be the same in insects as in all other animals that have been investigated, and capable of interpretation in terms of the ionic hypothesis, as developed on the basis of studies with other animal groups. According to this hypothesis the capacity for conduction should be markedly influenced by the proportionate concentration of inorganic ions in the medium that bathes the nerve fibre; high concentrations of potassium in the extracellular fluid should tend to reduce the magnitude of the resting potential, while low concentrations of sodium should reduce the positive overshoot of the action potential, on which the propagation of the impulse depends. These general properties of excitable tissue are of particular interest in relation to nervous function in insects, in view of the wide variation in haemolymph composition which characterizes different members of the group (see Chapter 5). In most species the Na^+/K^+ ratio is substantially different from the value of about 10 which characterizes vertebrate body fluids, and in some of the phytophagous members the concentration of potassium and magnesium may actually exceed that of sodium, yet nervous function seems to be in no way impaired.

The occurrence of a characteristic sheath investing the nerves and ganglia of insects was seen to be of possible significance in relation to this problem, and the structure of the sheath and of its associated cellular elements has come under intensive investigation. The sheath itself, called the neural lamella, constitutes a substantial investment measuring as much as 5μ in thickness, and it is made up of a number of distinct layers, as shown in Fig. 8.1(a). These include an outer amorphous region, a narrow fibrillar layer in the middle, and an inner layer in which tangentially disposed fibres of a collagen-like material lie imbedded in a homogeneous matrix. Underneath the neural lamella is a cellular layer called the perineurium, the cells of which are characterized by massive accumulations of glycogen granules, and by the occurrence of clusters of elongated mitochondria.

In view of the fibrous structure of the neural lamella there can be little doubt

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(a)

(b)

Fig. 8.1. The neural sheath of insects. (a) Schematized cross-section of the nerve sheath and perineural layer in the cockroach. a, amorphous layer; b, zone of fine filaments; c, fibrils of collagen-like substance in the main region; d, clusters of mitochondria; e, granules believed to be glycogen; f, nucleus of perineural cell. (Drawn from electron micrographs of Smith and Treherne, 1963.) (b) The escape of ^{22}Na from the nerve cord of the stick insect during washing with non-radioactive physiological saline. Closed circles, total exchange; open circles, fast component of the total exchange, obtained by subtraction of exponential portion extrapolated to zero time (Treherne, 1965a).

that it serves the function of mechanical support for the nervous system which it invests. The occurrence, however, of a cellular layer, with a submicroscopic structure that suggests a high level of metabolic activity, would indicate that the complex plays a more active role than that of support. The possibility that it

might function to maintain the inorganic composition of extracellular fluids at a level suitable for nervous function has been investigated by Treherne, whose work forms the basis of the discussion that follows. He demonstrated first of all that inorganic and organic ions exchange quite readily between central nervous system and haemolymph. The exchange occurs as a two-stage process, with an initial rapid phase giving way to a slow, exponentially decaying, exchange. This is shown in Fig. 8.1(b), where the loss of radioactive sodium, from a nerve-cord previously loaded with the isotope, is depicted as it occurs during washing in non-radioactive saline. The evidence suggests that the initial flux represents an exchange between haemolymph and the extracellular fraction of the nervous system, and determination of the corresponding extracellular volume has enabled estimates to be made of the composition of extracellular and intracellular fluids for comparison with that of the bathing fluid. It can be seen from Table 8.1 that there are marked differences between the external medium and the extracellular compartment, and in both species the extracellular fluid shows an elevated Na^+/K^+ ratio. In the cockroach, the difference can be accounted for on the basis of a simple Donnan equilibrium between external and extracellular compartments across the nerve sheath; this is the reason why removal of the nerve sheath results in a change in the composition of extracellular fluids, and why de-sheathed preparations are rapidly depolarized by high potassium concentrations in the external medium, while in normal preparations the rate of depolarization is much lower. In the stick insect, however, the distribution of cations between the two compartments cannot be interpreted on the basis of a simple Donnan equilibrium. Here the relatively high concentration of sodium in the extracellular compartment was found to suffer substantial reduction in the presence of metabolic inhibitors, and it was concluded that an active process was involved in the maintenance of this difference in concentration across the neural sheath.

Despite the existence of such an active process, the ionic ratio of the extracellular fluid is far from being equivalent to that of the body fluids in other animal groups, and the question remains whether its composition is in fact compatible with the conduction of nerve impulses as interpreted by the ionic hypothesis. On the basis of the equilibrium potentials for potassium and sodium, as set out in the last column of Table 8.1, it can be seen that the conditions for impulse conduction along the lines of classical membrane theory would, in fact, be fulfilled, albeit on the basis of a rather low level of resting potential (-37 mV as compared with -64 mV for the cockroach and -70 mV for vertebrates in general), and with the potentiality for positive overshoot at the peak of the action potential, as given by the sodium potential, substantially reduced ($+22.3$ mV as compared with $+35.8$ mV for the cockroach and $+60.0$ mV for vertebrates).

It may be concluded that the apparent anomaly posed by the specialized

TABLE 8.1

The distribution of sodium and potassium between the external medium and extracellular and intracellular compartments of the nerve cord of the cockroach and the stick insect (from Treherne, 1965b)

	External	Extracellular	Intracellular	mV Equilibrium potential
		mmoles/l		
Cockroach				
Na^+	158	284	67	+35.8
K^+	12	17	225	−64.0
Na^+/K^+	13	17	−	
Stick Insect				
Na^+	20	212	86	+22.3
K^+	34	124	556	−37.1
Na^+/K^+	0.6	1.7	−	

composition of the haemolymph in certain groups of insects may in all probability be resolved on the basis of Treherne's investigations. The nerve axons themselves appear to be maintained in an environment which is compatible with impulse conduction by virtue of the presence, and in some cases of the activity, of ensheathing elements.

2. Neuromuscular Transmission

The small size of insects would be likely to raise a special problem in the context of neuromuscular activity, because many of the muscles on whose activity movement is based are minute, and the reduction in size of the motor apparatus is achieved by a reduction in the number of muscle fibres rather than by a reduction in the size of the fibres. The problem of producing a finely graded contraction, on which well co-ordinated movement must depend, is therefore not capable of solution on the basis of the familiar vertebrate pattern, where each muscle is divided into a number of motor units, and the contraction of the muscle as a whole is based on the recruitment of motor units, each of which contracts maximally. In insects, where muscles may contain no more than one or two fibres, a mechanism must be provided whereby the contraction of each individual fibre can be graded over a wide range, and interest will centre on the means by which this is achieved.

a. Anatomy of the Motor End-Plates

Certain anatomical features of muscle innervation in insects are of obvious relevance in relation to the problem of the graded response. In the first place,

each muscle fibre is provided, not with a single end-plate as in vertebrate fibres, but with a series of end-plates, distributed at regular intervals over the whole surface of the fibre, with a spacing of between 40 and 80 μ, and with each motor end-plate supplied by its own branch of the motor nerve. Secondly, a proportion of the muscle fibres in the muscles of many insects are dually innervated, each neuromuscular junction supplied by two separate axons, as illustrated in Fig. 8.2. One of the axons is designated as the "slow" axon, the other as the "fast", on the basis of their physiological performance which will be described below. Whether singly or dually innervated, the motor end-plate typically takes the form of a claw of branching axon-terminals, sunk into gutters on the surface of

Fig. 8.2. Schematic drawing of the structure of a typical insect neuromuscular junction. The two motor axons travel together inside a common sheath, from which they both emerge at the point of contact with the muscle fibre, where the basement membrane of the muscle fibre fuses with the neural lamella of the nerve. The terminal branches fit into simple grooves on the surface of the muscle fibre, accompanied by the cells of the neural sheath (Hoyle, 1965).

the muscle cell, often accompanied by tracheolar branches. The axon membrane comes into intimate contact with the muscle membrane, and synaptic vesicles have been shown to be associated with the presynaptic membrane. By analogy with the vertebrate pattern it may be presumed that these presynaptic vesicles contain the neuromuscular transmitter.

A third axon has been shown to innervate a proportion of fibres in the tibial extensor of the jumping leg of the locust, and the possibility that this axon may be comparable to the inhibitory fibre of the Crustacean system has been considered. It has not so far been possible to correlate activity in this axon with unequivocal mechanical effects in the isolated preparation, but if activity is monitored in unrestrained animals it is found to coincide with flexion of the tibia, lending support to the view of an inhibitory function. However, detailed discussion of the physiology of this fibre would be out of place, in view of the uncertainty concerning its precise role.

b. Physiology of the Motor End-Plates

The physiology of neuromuscular transmission appears to be basically the same in insects as in other animals, involving a release of transmitter substance from presynaptic nerve terminals, triggered by the arrival of impulses in the motor nerve. Miniature end-plate potentials have been detected in unstimulated insect preparations, and by analogy with the situation in vertebrates, they have been interpreted as caused by the spontaneous release of transmitter quanta from the presynaptic membrane. The difference between insects and vertebrates appears to lie not so much in the mechanism of transmission, as in the nature of the transmitter. While acetyl choline has been firmly implicated as the transmitter of the vertebrate neuromuscular junction, attempts to establish the occurrence of cholinergic transmission in insects have proved unsuccessful. It is only recently that a likely candidate for the role of transmitter has appeared, in the form of glutamic acid. This substance is capable of causing depolarization of the motor end-plate in insects at physiological concentrations, and it appears that presynaptic stimulation causes the liberation of glutamic acid from *in vitro* preparations. It is a little surprising to find an amino acid of such central metabolic importance (see Chapter 1), and a substance so widely distributed and occurring in such high concentrations in haemolymph and muscle fluids alike, involved in the special role of neuromuscular transmitter. The apparent anomaly cannot be accounted for on the basis of a sealing-off of receptive regions from the haemolymph, since the presence of glutamic acid in low concentration is capable of causing tonic contraction in perfused preparations, indicating that the substance gains ready access to the receptor surface. The possibility that the concentration of "free" glutamate in the haemolymph may be much lower than would appear from standard amino acid analyses has been raised, but experimental evidence is lacking.

(i) The Slow Response. Stimulation of the slow axon with single shocks causes depolarizations of the muscle membrane ranging in magnitude from 2-30 mV, depending on the particular site from which recording is made. Little mechanical activity is associated with single shock stimulation, but if the preparation is stimulated with a train of impulses at the rate of 5-20 stimuli/s, the end-plate potentials show considerable facilitation (Fig. 8.3(a)), and a slow

Fig. 8.3. Slow and fast responses of the tibial extensor muscle of the locust. (a) Electrical responses: stimulation of the fast axon gives a large, non-facilitating end-plate potential (x) on which is superimposed an active membrane response (y); stimulation of the slow axon gives a small end-plate potential, which shows a progressive increase in size during repetitive stimulation, but does not initiate an active response. (b) Mechanical response of the slow muscle fibres: with increasing frequency of stimulation, as indicated above each trace, the velocity and extent of contraction increases. (Redrawn from Hoyle, 1965.)

mechanical response is evoked. The greater the frequency of stimulation the greater, usually, the degree of facilitation; the development of tension is correspondingly faster and the peak tension attained greater, up to a limit at about 80 stimuli/s (Fig. 8.3(b)).

(ii) The Fast Response. Stimulation of the fast axon with single shocks produces a large end-plate potential with an electrically excited spike component (Fig. 8.3(a)), and the extent of depolarization is not facilitated by repetitive stimulation. The positive overshoot is not of sufficient magnitude, in the normal fibre, to ensure all-or-none propagation, so the response is conducted decrementally from the motor end-plate. The mechanical response to a single shock is a well-developed twitch; the twitch contractions fuse at about 10 stimuli/s, but the mechanical response is greatly facilitated at higher frequencies, to give tetanus/twitch ratios of as much as 10.

c. The Graded Response

On the basis of the anatomical and physiological results discussed, one can visualize, in general terms, how insects have solved the problem of producing an accurately graded response from muscles which comprise a minimum of fibre units. The basic approach has been through a graded electrical and mechanical response, as opposed to the all-or-nothing response that characterizes the vertebrate system. If we consider a muscle like the extensor of the locust jumping leg, it is clear that a full range of tensions from zero to maximal can be elicited by suitable patterns of activation in the slow and fast axons, with the lower range of tensions and contraction velocities catered for by tetanic discharge of the slow motor neurone at increasing frequencies, the higher range by superposition of fast axon activity, operating on the basis of mechanical facilitation at peak tension. Since all electrical events are essentially local, the multi-terminal innervation is a necessary condition for maximal activation of the contractile machinery. It should be mentioned that in a number of insect muscles, some opportunity for gradation based on subdivision of the muscle into motor units exists, side by side with the capacity for gradation in individual units. The tibial flexor of the locust, for example, is innervated by three fast motoneurones which supply different parts of the muscle, so that gradation of tension development by recruitment of active fibres is a possibility.

d. Flight Muscle

Before leaving the subject of neuromuscular transmission some account must be given of the activation of flight muscles in insects with so-called "asynchronous" or "indirect" flight musculature. The distinction between synchronous and asynchronous flight musculature was originally based on the observation that while the flight of insects like the locust, with wing-beat frequencies of about 20/s, could be adequately accounted for on the basis of known properties of the units of neuromuscular function (e.g. conduction velocity, latency of neuromuscular transmission, latency of contraction, contraction time and relaxation time), problems arose with certain insects for which wing-beat frequencies in excess of 1000/s have been recorded. On the basis of the anatomy of the pterothorax it was clear that each wing-beat cycle involved the contraction of antagonistic muscle pairs, acting as levators and depressors. With a wing-beat frequency of 1000/s, the time for a complete cycle would be 1 ms, and for the contraction and relaxation of one of the pair of antagonists 0.5 ms. When it is considered that a limiting factor for one of the fastest links in the chain of events, namely conduction, is the absolute refractory period of impulse conduction which exceeds 1 ms, it is clear that impulses could not be fired at the requisite frequencies in motor nerves to the flight muscle, much less could the activation of normal contractile machinery be accomplished in the requisite time. This startling anomaly prompted a thorough investigation

(i)

(ii)

(a)

(b)

(c)

Fig. 8.4. Neuromuscular aspects of insect flight. (a) A comparison between electrical (upper traces) and mechanical (lower traces) records from the thorax of (i) a butterfly (synchronous), and (ii) a blowfly (asynchronous), during flight (Pringle, 1965). (b) A. Simplified model of an insect thorax: pressure in the direction of the arrow produces a click action only if there is an inward force from the sides of the model. B. Relationship in the model between vertically applied force and wing deflection: curve a, with no lateral stiffness; curves b and c, with increased lateral stiffness; E, equilibrium position of model as shown in A (Pringle, 1965). (c) Effect of small sudden changes of length on the tension developed by beetle fibrillar muscle. While developing a steady tension of 20 g, the muscles are quick-stretched and quick-released by various amounts at 0 and at 20 ms (Pringle, 1965; from Boettiger, unpublished).

by Pringle, who discovered that contractions of the flight musculature in insects of this kind did not bear a one-to-one relation to the firing of impulses in the motor nerve. Figure 8.4(a) shows a simultaneous comparison of electrical and mechanical events in the flight musculature of insects with synchronous and asynchronous flight musculature. In the butterfly (synchronous), contraction of flight muscles can be seen to be associated regularly with depolarizations of the muscle membrane, indicating a one-to-one relationship of contraction to the arrival of motor impulses, at a rate of about 50/s. In the blowfly, wing-beat frequency is about 160/s, but the rather irregular electrical events bear no relation to the mechanical events, and occur at a very much lower frequency. It appears that the asynchronous muscle is capable of going through a succession of contraction cycles under the influence of a single motor impulse, suggesting that the coupling between electrical and mechanical events is quite different from what it is in normal muscle.

Further investigation of the mechanisms involved in the flight of such insects revealed the existence of a peculiar mechanical system in the pterothorax. The flight muscles do not engage directly on the base of the wing, as they do in insects with direct flight musculature. Instead they extend dorsoventrally and longitudinally across the rigid box of the pterothorax, their contractions causing deformations of the mechanical structure that are transmitted to the wings in such a way that activation of the dorsoventral muscles causes wing elevation (see Fig. 8.4(b)), while the longitudinal muscles serve to depress the wings. The nature of the articulation and of the mechanical system is such that a "click"-mechanism is involved; for example, when wings are in the depressed position, and a force is applied to mimic contraction of dorsoventral muscles, elevation of the wings meets with increasing resistance until the wings click over abruptly into the elevated position (see curve c of Fig. 8.4(b)). The degree to which this phenomenon is manifested depends on the lateral stiffness of the thorax (cf. curve a), which in many insects is reinforced by special pleurosternal muscles.

The development of a type of "click"-mechanism is not, in fact, confined to insects with indirect flight muscles, but occurs also in species like the locust whose wings are directly activated. In all cases it ensures that a large proportion of the force of muscular contraction is taken up by the elasticity of the antagonistic muscles and by the cuticular structure of the thorax and wing articulation, and thus becomes available for moving the wings in the opposite direction at the end of a wingstroke. The development of a special elastic protein, resilin, in association with the wings of insects is of special significance in relation to the efficient storage of kinetic energy developed during the wingstroke, accounting for nearly a third of the energy stored in the locust. It is, however, in relation to the flight of insects with indirect musculature that the development of "click"-mechanisms becomes of special significance; for when

the muscles have contracted up to a certain point (the point of equilibrium in Fig. 8.4(b)), there will be a sudden release of tension as the wing clicks into its new position of stability. This release seems to inactivate the muscle, but at the same time the tension which is suddenly applied to its antagonist causes that to develop active tension. The over-all result is a rapid alternate contraction of the antagonistic muscle units, the system behaving as a resonant mechanical oscillator, with the rate of oscillation determined principally by the mechanical properties of the pterothoracic box. It appears that the function of motor impulses, which arrive at the rate of one for every 4-10 oscillations, is to maintain the muscle fibres in an active state, capable of responding to the application of force in the way described.

Detailed investigation of these asynchronous (or fibrillar) muscles at the level of the contractile machinery have been undertaken by Pringle and his collaborators, using the large flight muscles of tropical beetles and bugs. These muscles show a characteristic response to externally applied load differing from that of normal muscle. Generally when muscle is subjected to a decrease or an increase in length during tetanic contraction, there is a transient rise, or fall, in tension, and the muscle then settles to a steady level characteristic of the new length. With fibrillar muscle, on the other hand, the immediate transient effects are followed by a further rise, or fall, after a brief delay (see Fig. 8.4(c)). In other words, there is a marked influence of the change in length on the contractile machinery of fibrillar muscle, and this appears to constitute its peculiar property, on which the contraction of asynchronous flight muscle is based. Further work has shown that the capacity for oscillation of the type described is a property of the contractile machinery as such, since muscle fibres from which most of the enzymatic and associated machinery has been removed by glycerination are capable of entering upon activity of this kind in the presence of ATP.

3. Synaptic Transmission

The unit of integrative activity in insects, as in other animals, is the synapse, and the question arises whether peculiarities of insect organization, including the limitation set by small size and the correspondingly small number of nervous elements at their disposal, might be reflected in corresponding peculiarities at the level of synaptic transmission. Before this problem can be considered it will be necessary to give some account of the extremely complex submicroscopic structure of the central nervous system of insects, which has been the subject of intensive investigation during recent years.

The neural elements of which the central nervous system is made up are basically of three kinds:

(a) the axon terminations of sensory cells conveying the input of information

which serves as the raw material of integrative activity. The bipolar (type i) or multipolar (type ii) cell bodies of these neurones are situated at the periphery, in close association with the sense organs which they innervate;

(b) the unipolar interneurones, confined entirely within the central nervous system, and mediating between the sensory input and the motor output; and

(c) the motoneurones, whose cell bodies are located in the central nervous system, with an axon emerging from it to supply the muscles and effector organs of the body.

Associated with these neural elements in the central nervous system are numerous glial cells, of which two general types can be recognized (three, if the perineurium is included):

(a) those associated with the cell bodies, or perikarya, of the central nervous system; and

(b) those associated with the neuropile, the region of the central nervous system where axon collaterals of motor and internuncial neurones meet and mingle with the terminal branches of sensory fibres.

The general disposition of these elements is shown in Fig. 8.5(a), which represents a cross-section of part of an insect ganglion, ensheathed by neurilemma and perineural layers. The perikarya, with their associated glial cells, are situated peripherally in the ganglion, with the neuropile central, the two separated by a layer of glial cell bodies. The corresponding submicroscopic structure, as revealed mainly by the electron microscope, is one of enormous complexity, but the main features are made clear in the simplified diagram of Fig. 8.5(b).

Beneath the neurilemma and perineurium, which have been dealt with in an earlier section, lies the outer layer of the ganglion, composed of the cell bodies of motor and internuncial neurones together with the proximal parts of axons originating from them, and their associated glial cells. The anatomical relationship between them affords some clue to the role of glial cells in this region, for the cell bodies of the neurones are deeply indented by cytoplasmic processes of the glial cells to form the so-called trophospongium. The tremendous increase in the area of contact between perikaryon and glial cell suggests that the arrangement mediates an interchange of material between the two types of cell, and lends support to the view that the glial cells are concerned with the transfer of nutrients from the haemolymph, or from the fat bodies which often ensheath central nervous ganglia, to the ganglion cells. The glial cells, like those of the perineurium, contain numerous minute granules which are believed to be composed of glycogen, and a transfer of glycogen and lipid material from glial cell to the neurone perikaryon is suggested by the work of Wigglesworth (1960). It seems that this special mechanism of cellular nutrition may be a reflection of the lack of haemolymph circulation within the tissues of the ganglion; nutritive and excretory exchanges would lack the advantage of bulk flow in a circulating

medium, and would need to be subserved by special mechanisms of active cellular transfer in the body of the organ.

A second characteristic feature of the outer glial layer is the extensive system of extracellular lacunae which is interposed between the ensheathed cell bodies and axons (see Fig. 8.5(b)). These lacunae often appear empty in electron microscope preparations, but usually they contain an electron dense material which may be a mucopolysaccharide. There can be little doubt that this system of lacunae represents the anatomical counterpart of the fast-exchanging extracellular space which has been described in an earlier section of this chapter, and it is possible that the anionic groups of the mucopolysaccharide which it appears to contain may act as a cation reservoir and thus play a part in the maintenance of a high extracellular sodium concentration.

The last feature of the outer glial layer which deserves mention is the sheathing of major axons which occurs in this region, by envelopment of the nerve fibres in folds of the glial cells, to form the so-called mesaxon (see Fig. 8.5(b)). This presumably serves to insulate the axon from presynaptic influences in this outer region of the ganglion, so that it is not until it reaches the neuropile that synaptic interactions can take place.

Interspersed between the outer glial layer and the neuropile is the second layer of glial cells, from the surface of which cytoplasmic processes extend to accompany the axons as they enter the neuropile. This produces a highly complex interdigitating network of cellular extensions, and the prospect of elucidating functional pathways within this network are remote. All that can be said at present is that axon profiles in the neuropile are often separated by the interposition of glial processes, and it may be presumed that under these circumstances synaptic interactions are likely to be precluded. A proportion of profiles, however, can be seen to be closely juxtaposed without intervention of glial membranes (see Fig. 8.5(b)) and it is often possible to distinguish what, by analogy with vertebrate systems, may be presumed to be presynaptic fibres containing aggregations of synaptic vesicles, from postsynaptic fibres whose cytoplasm is empty of such. It is not thought that such juxtaposition necessarily implies functional interaction, but in many regions a clustering of vesicles is associated with a thickening of both presynaptic and postsynaptic membranes, and these differentiated parts are likely to constitute active sites. What can be said, therefore, in general terms, is that the neuropile represents a part of the central nervous system where the transmission of excitation between axons of different neurones can be accomplished, and that the pattern of transmission, which forms the basis of integrative function, will be governed in part by the degree of overlap between axons from different neurones, and in part by the pattern of interposition of glial processes; these may be visualized as dissecting the neuropile into functional pathways, so forming a substratum for integration.

These studies of the submicroscopic anatomy of the central nervous system

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of insects have indicated that the fundamental basis of transmission is likely to be similar to that which has been experimentally established for other animals; they would then involve the release of chemical transmitters from presynaptic fibres, and the diffusion of such transmitters across narrow synaptic clefts to receptor sites on the postsynaptic membrane, where they would exert an effect based on alterations in membrane permeability and hence of membrane polarization. But while the basic mechanism of synaptic transmission may be the same, differences in the pattern of organization of synaptic interaction must be presumed to reflect important differences in the details of integrative function. In vertebrates the excitatory and inhibitory postsynaptic potentials are produced at synapses which encrust the perikaryon and its dendrites, and which can

Fig. 8.5. The cellular structure of the central nervous system of insects. (a) Schematized plan of a section through an insect ganglion. a, neural sheath; b, perineurium; c, peripheral layer of perikarya with associated glial cells; d, inner layer of glial cells, associated with e, the neuropile; f, motor nerve; g, muscle fibre; h, sensory nerve; i, sense organ; 1, motoneurone cell body; 2, interneurone cell body; 3, sensory neurone cell body. (b) Diagram illustrating the disposition of cellular and extracellular components in an insect ganglion. Glial cytoplasm is indicated by light stippling, and extensive extracellular spaces by dark stippling. NL, neural sheath; PN, perineurium; OG, layer containing perikarya and associated glial cells, with an inner layer of glial cells associated with the neuropile, NP; ax_1, nerve fibre surrounded by concentric glial sheath; axon profiles 2-10 ensheathed by glial cells; other axon profiles closely apposed without glial separation, as shown at arrows. Large interconnecting extracellular spaces are present between the glial cell bodies and between their neuropile extensions, especially in the neighbourhood of tracheoles, tr. Note that the width of the region between perineurium and neuropile has been reduced for purposes of clarity, while the size of extracellular spaces between axon branches and glial processes in the neuropile has been exaggerated (Smith and Treherne, 1963).

Fig. 8.5(b)

therefore be focussed on to a particular region of the neurone, the initial segment, where impulse initiation occurs. In insects one must imagine that the necessity to provide for the nutrition of the perikaryon by ensheathing glial cells would preclude the use of the cell body for synaptic transmission, so that this process has had to be relegated to sites of interaction on the axon itself or on

axon collaterals. The profuse branching which characterizes the axons of insects would entail a wide spacing of sites of synaptic interaction, and this would militate against the use of a single focus for impulse initiation; it seems likely that impulses may, in fact, be generated at any one of a number of sites along the branched structure. In the absence of specific electrophysiological information one could speculate that such multiple impulse initiation may be of special

A → 1   D → 3,4,5
B → 1,2,3,4,5   E → 5
C → 3

(a)

(b)

Fig. 8.6. Interneurones in insects. (a) Diagram of an interneurone, to illustrate how the output from the neurone might depend on the site of input. A-E, input sites; 1-5 outputs (Hughes, 1965). (b) The fourth giant internuncial from the central nervous system of *Gerris* (Hughes, 1965; from Guthrie).

significance in relation to the necessity for insects to effect nervous integration on the basis of a relatively small number of functional units. Figure 8.6(a) indicates the way in which different parts of a single neurone of the type described could function independently of each other, with impulses initiated at different points on the branched system depending on the nature of the input, and with the output governed in part by the site of impulse initiation, and in part by the ability of an impulse initiated in one region of the branched system to invade other regions, which could be a simple function of the relative diameter of branches. In this way the output from a neurone could take a number of different forms, and could be directed to a number of different parts of the nervous system, according to the pattern of input, and a single neurone could thus perform the function which on the vertebrate pattern would need a number of separate neurones. The bizarre configuration of certain insect interneurones (see Fig. 8.6(b)) suggests that the potentialities for this kind of effect may be considerable.

SENSE ORGANS

The motor system and the central nervous system of insects have been shown to be fashioned from relatively small numbers of cellular elements, and the same applies in the sensory field. The density of sense organs on the surface of an insect's body is several orders of magnitude smaller than that associated with the body surface of a mammal; and where specialized receptor regions are concerned, as in the eye, the number of sense organs involved in insects is counted in thousands, in mammals in millions.

Certain other characteristics of the sensory physiology of insects appear to be referable to the possession of a rigid exoskeleton. This would constitute a formidable barrier to the more attenuated forms of environmental energy, and where such are concerned, as in olfaction and gustation, holes in the integument have had to be provided to allow the stimuli direct access to sensory elements. The monitoring of stresses set up in such a rigid exoskeleton has seemed to provide another special problem, solved by the development of sensilla which are sensitive to the shear forces arising as a result of mechanical deformation. Photoreception has remained relatively unaffected by the existence of a rigid exoskeleton, since the property of rigidity is not inconsistent with that of optical transparency.

a. Photoreceptors

Sensitivity to visible light is associated with two main receptor regions, the simple eyes, or ocelli, and the compound eyes. The structure and function of the compound eyes of insects have occupied the attention of insect physiologists for many decades, but despite the great amount of effort which has been devoted to the problem of insect vision, and despite the advances which the development of electron microscopy and the refinements of electrophysiological techniques have made possible, the physiology of insect vision remains poorly understood. The hypotheses of early physiologists concerning the mechanism of image formation have been put in question by recent experimental work, and lively controversies have developed on the subject of the system's optical properties, while the fundamentals of the photoreceptive process itself remain as yet completely

unknown. It would clearly be inappropriate, in a book such as this, to attempt a detailed exposition of so controversial a field, and the present account will for this reason be a relatively superficial one.

(i) The Structure of the Compound Eye. The compound eyes of insects are made up of structural units called ommatidia, whose numbers range from a dozen or so to several thousand in different species. Each ommatidium may be considered to be made up of three functional parts (see Fig. 9.1(a) and (b)):

(a) the dioptric structures, which comprise a transparent part of the cuticle, known as the cornea, and a deeper-lying crystalline cone, through which light penetrates to the sense organs beneath;

(b) the photosensitive region of the ommatidium, known as the retinula, and composed usually of eight retinular cells. Each cell is a primary sensory neurone, continuous with a nerve fibre which passes, through the basement membrane that supports the sensory cell, to the central nervous system. The retinular cells are grouped around a central axis, and their photoreceptive regions, known as rhabdomeres, are centrally juxtaposed and sometimes fused to form an axial

Fig. 9.1. The structure of the compound eye. (a) The ommatidium of a cockroach, and (b) of a moth in the dark-adapted and light-adapted states, showing migration of pigment, nuclei and cytoplasm. b.m., basement membrane; b.ret.c., basal retinular cell; cor.l., corneal lens; cor.p.c., corneal pigment cell; cn., crystalline cone; ret.c., retinular cell; ret.c.n., retinular cell nucleus; rhb., rhabdom; sec.p.c., secondary pigment cell; tr., trachea (Goldsmith, 1964, after Hesse; Umbach; and Day). (c) Cross-section of the retinular cells of the bee. M, mitochondrion; N, nucleus; P_1, pigment granules of retinular cells; P_2, pigment granules of pigment cells; R, rhabdom (Goldsmith, 1964).

rhabdom (see Fig. 9.1(c)). Closely packed arrays of microtubules are aligned at right-angles to the axis of each rhabdomere; and

(c) the pigment cells, which contain granules of red, yellow or brown pigment, and form a sheath round each ommatidium.

Two main kinds of ommatidia can be distinguished in different species of insect. In those that have the so-called "apposition" eyes, the rhabdom extends the full length of the ommatidium from basement membrane to crystalline cone, as in Fig. 9.1(a), and the distribution of pigment in the pigment cells is little affected by conditions of illumination. "Superposition" eyes occur typically in species that are nocturnal or crepuscular, and here the rhabdom is confined to the basal half of the ommatidium, and the distribution of pigment varies greatly depending on illumination (see Fig. 9.1(b)). In the dark-adapted eye the pigment granules aggregate distally in the pigment cells, while in the light-adapted condition they are more uniformly distributed between cornea and tip of rhabdom (for further details see (iv) below).

After passing through the basement membrane, the sensory axons enter the optic lobe of the brain, where they establish complex synaptic connections in a series of neuropiles designated as the first, second and third optic ganglia (Fig. 9.2). Two kinds of retinular axon can be distinguished, one of which has short

Fig. 9.2. Diagrammatic representation of neural connections in the eye and optic lobes of an insect. a, neurones associated with one synaptic region; cn, connecting neurones within the optic lobe; lvf, long visual fibre; nn, neurones connected to the central nervous system; svf, short visual fibre; I, II, III, first, second and third synaptic regions (Burtt and Catton, 1966).

fibres that terminate in the first optic ganglion, and establish synaptic contact with the lateral branches of multipolar interneurones. There appears to be much convergence in this region, each ganglion cell receiving input from sensory cells in neighbouring retinulae. Fibres from the second kind of retinular cell pass straight through the first ganglion and, accompanied by axons from the monopolar interneurones of that ganglion, they enter the second ganglion to establish synaptic contact with interneurones whose fibres pass to the central nervous system, as well as with interneurones that relay to the third ganglion. In addition to these centripetal pathways, there are centrifugal fibres originating in cell bodies deeper in the brain and passing outwards to branch profusely in the neuropile of the first ganglion.

(ii) Pigments of the Compound Eye. One of the most characteristic features of the insect eye is its heavy pigmentation, with pigment granules clustering not only in the special pigment cells, but also in the retinular cells themselves (see Fig. 9.1(c)). None of these pigments, however, appear to be directly involved in photoreception, since it has been shown that white-eyed mutants of several species of insect, which contain no trace of such pigments, are as sensitive to light as are the normal wild type. It seems likely that it is vitamin A and its aldehyde, retinene, both of which have been isolated from the heads of insects, that constitute the actual visual pigments in these as in other animals, the quantities involved being minute by comparison with those of the accessory pigments. These latter appear to function primarily as light shields, preventing stray light from moving obliquely through the eye, and they are of three main types, the ommochromes, the ommatins and the pterins (see Chapter 1). They have peak absorption in different regions of the spectrum, so that in combination they form an effective barrier to all but the longest wavelengths. The photolability of some of these pigments was originally thought to indicate a direct role in photoreception, but it now seems likely that if the phenomenon has biological significance it is in relation to the regulation of the amount of light that reaches the photoreceptive structures.

(iii) Electrophysiology of the Compound Eye. Because of the very small size of the retinular cells of the compound eye, it is only recently that progress has been made on the recording of unit events. Early investigators had to content themselves with investigations of the massed response to light of whole sheets of sensory cells, recorded in the form of a so-called "electroretinogram". Under these conditions a depolarizing response to light is obtained, which at low intensities is represented by a simple negative plateau, while at high intensities there is an initial fast phase which overshoots the negative plateau. The magnitude of the plateau is a close function of stimulation intensity. These potential changes appear now to be the expression of similar effects at the level of individual receptor cells, the responses of which are illustrated in Fig. 9.3. In view of the magnitude and positive sign of these potentials there can be little

Fig. 9.3. Depolarizing potentials, presumably of a retinular cell, in the compound eye of a bee, recorded with intracellular electrodes during stimulation of the eye by light of different intensity, decreasing from A to D. Duration of stimulus 0.9 s (Goldsmith, 1964 from Naka and Eguchi).

doubt that they are developed across the membrane of the retinular cell, but the precise site of their origin has not yet been unequivocally established. Spike potentials have also been recorded by intracellular electrodes in the retinular cells, apparently initiated at the cell base or in the axon itself.

Measurements of the latency of spike responses recorded at various points in the optic pathway between the retina and the ventral nerve cord following stimulation by light suggest that, at moderate intensities, the excitation is relayed successively through each of the three optic lobes, but with stronger stimuli some short-circuiting of synapses in the second and third ganglia occurs, to give a reduced latency. Unit activity of elements in the second ganglion have been investigated, and a high proportion of cells were found to give bursts of spikes at light-on and light-off. Evidence was obtained of substantial convergence, some units responding to illumination of any point in the visual field.

(iv) Light and Dark Adaptation. In the compound eye, as in photoreceptors of other types, there is a decrease in the sensitivity to light during exposure to light (light adaptation), and a recovery during darkness following such exposure (dark adaptation). In insects with apposition eyes, where there is little movement of pigment, dark adaptation proceeds to completion within 20 min, the initial stages of recovery being very rapid. In insects with superposition eyes, the curve has an initial rapid phase, followed by a much slower phase of recovery, as illustrated in Fig. 9.4. The initial phase is similar to the recovery of sensitivity in apposition eyes, and may be attributed to some process of recovery in the photosensitive process whose precise nature has not yet been identified, but which may involve a resynthesis of visual pigment. The second, slower phase is closely associated with, and appears to be caused by, migration of the accessory pigments in the pigment cells (see inset of Fig. 9.4). This is confirmed by the fact that in a certain proportion of individuals there is a failure of such pigment migration, and where this occurs there is failure, too, of recovery beyond the level of the first part of the curve.

(v) The Physiology of Vision. While there has been considerable progress during recent years in the exploration of fine structure and of unit electrophysiological correlates of photoreception, the complexity of the architecture of

Fig. 9.4. Dark adaptation (A) and pigment migration (B) in *Cerapteryx graminis*. Curve A derives from measurement of 22 preparations; curve B shows the composite results obtained from 90 preparations representing average values of measurements in 2-5 specimens. Pigment migrations are given as a percentage of the distance between extreme light (inset C) and dark position (inset D) of the proximal border of the secondary pigment (Goldsmith, 1964 from Bernhard, Höglund and Ottoson).

the compound eye and its associated neuropiles has so far precluded a convincing interpretation of the visual process in terms of the properties of its constituent elements. Behavioural experiments with a wide variety of insects have established the existence of well-developed form discrimination (see Chapter 11), but the physiological basis of this capacity remains obscure. A general theory of insect vision was put forward early in the history of investigations by Exner, who proposed that each ommatidium would be sensitive only to light entering at a small angle to its axis, other rays being absorbed by the screening sleeves of pigment cells. There would therefore be little overlap between the visual fields of adjacent ommatidia, and the image formed on the retinular layer of sensory elements would be essentially a mosaic. The situation would be slightly different in dark-adapted superposition eyes where, to ensure increased sensitivity, a peripheral migration of the pigment sleeves would allow light coming through one ommatidium to affect the photoreceptive regions of

adjacent ommatidia. Objections are currently being raised to this interpretation on the basis of recent experimental evidence, but no completely satisfactory alternative has so far been proposed. It therefore remains uncertain precisely what the nature is of the image which is projected on to the sensory elements, and what the mechanism is of its projection, nor is it known to what extent the receptive process may be under the control of centrifugal and lateral nervous influences analogous to those involved in vertebrate vision.

The situation in regard to another important aspect of the physiology of vision, namely colour vision, is rather more satisfactory. The existence of colour vision in insects was established a long time ago on behavioural criteria, and its electrophysiological basis has now been uncovered. It has been possible to change the spectral sensitivity curve of certain insects by adapting the eye to coloured light, and in this way the existence of two distinct visual pigments in the eye has been demonstrated, one with a peak at 340 mμ, and one with a peak at 540 mμ, thought to be located in different receptor cells. In flies it has been possible to investigate the spectral sensitivity of single sense cells using intracellular electrodes; all the cells tested showed a peak at 350 mμ, but while some had a second maximum at 450 mμ, in others the secondary peaks fell at about 480 and 520 mμ (see Fig. 9.5). The existence of different sensory neurones with different spectral characteristics of this sort is adequate to account for the existence of colour vision, as established on the basis of behavioural experiments.

(vi) Ocelli. Simple eyes occur in the adults of most winged insects, situated on

Fig. 9.5. Spectral sensitivities of three individual retinular cells of the compound eye of a fly, measured with an intracellular microelectrode (Goldsmith, 1964 from Burkhardt).

(a)

(b)

Fig. 9.6. Simple eyes of insects. (a) Section through the ocellus of *Arthrophora spurmaria*. c.n., nucleus of cornea-forming cell; l., lens; n.c., ocellar nerve; pc.n., nucleus of pigment cell; rh., rhabdom; sc.n., nucleus of retinula (Imms, 1948). (b) Potentials recorded from the ocellus of the cockroach in response to light of increasing intensity (top row) and in response to light of log intensity = −5 at different intervals during dark adaptation following light adaptation for 1 min at 12,000 ft candles. The duration of the test stimulus is shown on the lower trace of each record (Ruck, 1958).

the frontal region of the head between the compound eyes. They consist of an aggregation of light-sensitive cells, closely resembling the retinular cells of the compound eye, covered by a simple "corneal" lens of transparent cuticle (Fig. 9.6(a)). The short neurones converge to synapse below the retina with dendrites of a relatively small number of second-order neurones that extend from the brain in the form of an ocellar "nerve".

Stimulation of the ocellus with bright light causes depolarization of the retinular cells, the magnitude of the response being related to stimulation intensity and to the degree of dark-adaptation (Fig. 9.6(b)). The sense cell depolarizations in turn produce hyperpolarizing postsynaptic potentials which serve to silence the spontaneous discharge of the ocellar fibres during the period of illumination.

The role of ocelli has not yet been elucidated satisfactorily; in view of the great convergence there can be no question of image resolution, nor do the ocelli alone appear to be capable of mediating phototactic responses. It has been suggested that they may exert an indirect effect on visual and other reactions, by increasing the level of excitation of corresponding nerve centres, but the evidence is not convincing.

b. Mechanoreceptors

The mechanoreceptors of insects are characterized by a great diversity of anatomical form, and they have been classified in different ways by different authors. For present purposes some attempt must be made to reduce to simple terms what is in reality a most complex situation, and this has been done by considering insect mechanoreceptors as belonging to one of three main functional groups:

(i) mechanoreceptors which effectively extend the zone of contact between the insect and its environment, projecting from the insect's surface as some kind of tactile bristle;

(ii) mechanoreceptors which monitor the stresses set up in the exoskeleton itself, whether as a result of outside influences, like gravity, or of inside influences, like muscular contraction;

(iii) mechanoreceptors which register tensions within the body, arising usually as the result of movements of one part relative to another.

(i) Tactile Setae. The simplest type of mechanoreceptor is the tactile seta, whose structure is illustrated in Fig. 9.7(a). It consists essentially of a cuticular bristle articulated in a cuticular socket and innervated by a single bipolar sense cell. Associated with the sensillum are the trichogen and tormogen cells, responsible for the formation of the cuticular parts of the sensillum, and an ensheathing neurilemmal cell. The distal process of the neurone ends as a terminal filament, or scolopid body, at the margin of the socket, while its proximal axon connects to the central nervous system. Displacement of the setae

Fig. 9.7. Tactile setae. (a) Section through the base of a tactile bristle from the larva of a butterfly. a, base of hair; b, articular membrane; c, scolopoid body; d, sense cell; e, neurilemma cell; f, cuticle; g, trichogen cell; h, basement membrane; i, tormogen cell; k, vacuole; l, epidermis (Schwartzkopff, 1964 after Hsü). (b) Electrical responses of a mechanosensory hair from the blowfly to mechanical deformations of progressively smaller intensity (A, B and C). Arrows mark the beginning and end of stimulation; note that differences in the amount of deformation are reflected in the size of the generator potential, the size of the action potentials and the frequency of firing. Time marks at 0.2 s intervals (Dethier, 1963; courtesy, M. L. Wolbarsht).

causes mechanical deformation of the sensory process, and so sets up a graded generator potential which in turn initiates a discharge of spikes in the axon. The frequency and size of sensory nerve impulses are related directly to the magnitude of the generator potential, as indicated in Fig. 9.7(b).

While the basic anatomical structure of tactile setae is fairly constant, the precise nature of their response to stimulation shows considerable variation. At one extreme are the delicate tactile hairs which are so sensitive to stimulation that a gentle puff of air is sufficient to initiate discharge. These are generally of the "phasic" type, characterized by rapid adaptation; they give short, high-frequency bursts of impulses at the moment of deflection, but remain silent under conditions of constant deformation. At the other extreme are the stout bristles, whose displacement requires considerably greater force. These show a high rate of discharge during initial phases of stimulation, but the frequency drops to lower, steady levels under constant deformation. The rate of discharge of these "tonic" receptors often depends on the direction, as well as on the extent, of displacement.

(ii) Stress Receptors. Cuticular stresses are registered by a special type of sense organ known as a campaniform sensillum, which consists of a canal in the cuticle, covered by an elliptical, domed cap, and innervated by a single bipolar neurone, as illustrated in Fig. 9.8(a). The mode of action of these sense organs was established by the work of Pringle, who showed that tonic discharges could be initiated in the sensory nerve by suitable mechanical deformation of the cuticle, the discharge frequency depending on the extent of deformation (see Fig. 9.8(b)). Such deformations had the effect of increasing or decreasing the convexity of the dome, and hence of increasing or decreasing tensions acting on the terminal filament attached to it. Consideration of the geometry of the physical system suggests that the sensillum responds to the compression component of the shear force developed by mechanical deformation, and that the orientation of the sensillum will determine the orientation of shear force to which it is sensitive. This view receives support from a consideration of the distribution of sensilla (see Fig. 9.8(c)); the sense organs tend to be grouped into what would appear to serve as functional units, each sensitive to a different component of shear force, and hence to a particular form of mechanical deformation.

(iii) Stretch Receptors. The multipolar (type ii) neurones, which are widely

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Fig. 9.8. Campaniform sensilla. (a) Section through a campaniform sensillum from the cockroach. a, dome-shaped plaque; b, scolopale; c, vacuole in membrane forming cell; d, accessory cell; e, sense cell; f, neurilemma cell (Wigglesworth, 1965 after Hsü). (b) The pattern of discharge from campaniform sensilla on the maxillary palps of the cockroach stimulated by stronger (curve a) or weaker (curve b) stresses in the cuticle, produced by bending (redrawn from Pringle, 1938). (c) Details of the orientation of the six groups of campaniform sensilla on the third leg of the cockroach (Pringle, 1938).

(a)

(b)

(c)

0·5 mm

(a)

(i)

1 sec

(ii)

(iii)   (iv)

(v)   (vi)   (vii)

(b)

distributed among the internal organs of insects, have been intensively studied by Finlayson and Lowenstein, who were the first to establish the function of these structures in insects as stretch receptors. A number of different types have been described, some associated with strands of connective tissue, some with muscle, and some with specialized muscle fibres to form sense organs reminiscent of the vertebrate muscle spindle (Fig. 9.9(a)).

In the complete absence of stretch there is no discharge from these sense organs, but under slight stretch, which is the normal condition in the body, there is a low basal rate of non-adapting discharge at 5-10 impulses/s (see trace (i) of Fig. 9.9(b)), which continues steady for hours on end. Increasing tension leads to an increase in discharge frequency, which is maintained at the higher level, with frequency closely proportional to the degree of stretch. The receptors thus conform to the classical picture of the tonic receptor, but they are in fact capable of performing a dual function. Experiments involving alternate stretch and relaxation of the sense organs showed that discharge frequency under these conditions was a function not only of the degree of displacement, but also of the velocity of displacement (Fig. 9.9(b), traces (ii)-(vii)). With increasing frequency of displacement the tonic element of the discharge tends to drop out, and the fibre remains silent during periods of maximum displacement as well as during relaxation (traces (vi)-(vii)), possibly as a result of postexcitatory inhibition; at these frequencies (above 5 cyc/s) the receptors therefore behave in a phasic manner, monitoring frequency of stretch rather than degree of stretch.

Another type of mechanoreceptor that may respond to stretch is the chordotonal organ, consisting of one or more type (i) neurones, associated with two companion cells and slung between one point of the body wall and another. These sense organs appear generally to subserve a proprioceptive function, but in many cases they are associated with specialized auditory membranes for the reception of airborne vibrations (see Chapter 10 for further details).

This brief account of the mechanoreceptors of insects has done less than justice to the amazing variety of structure and function which is exhibited by this type of sense organ within the group. It must be emphasized, too, that the simple classification which has been adopted cuts across the physiological distinction which can usually be drawn between exteroceptors and proprioceptors. Examples from any of the three categories may function as either,

Fig. 9.9. Stretch receptors. (a) Diagram of the structure and innervation of a stretch receptor from the pupa of a moth *(Antheraea)*, showing the modified muscle fibres (upper) innervated by branches from a motor nerve, and the multipolar neurone, whose dendritic processes run alongside the muscle fibre on its lower surface (Finlayson and Lowenstein, 1958). (b) The response of a stretch receptor from the pupa of a moth to phasic stimulation; the upper trace in each record shows the neurone action potentials, the lower monitors mechanical displacement: (i) resting discharge; (ii) stretch at 0.5 cyc/s; (iii) at 1.5 cyc/s; (iv) at 2.0 cyc/s; (v) at 4.0 cyc/s; (vi) at 5.0 cyc/s; (vii) at 15.5 cyc/s; (schematized from oscilloscope photographs of Lowenstein and Finlayson, 1960).

depending on their location and the extent to which they are functionally coupled with similar units. For instance, while tactile setae normally could be considered as exteroceptive, in many insects they are grouped to form hair-plates near points of articulation of the exoskeleton, and under these circumstances they would function as proprioceptors, providing information about the relative position of parts of the body. Similarly, the stress receptors of the cuticle, while normally fulfilling a proprioceptive function, would register stresses imposed on the insect's body by gravity or the pressure of wind, and could in this sense be considered as exteroceptors; and chordotonal sensilla, whose primary function could be considered as proprioceptive, are often associated with accessory structures, as at the tympanic membrane or in the Johnston's organ of the antenna, and there serve a function of exteroception in relation to airborne vibrations. It is the very diversity of form and function of insect mechano-receptors that has necessitated a superficial treatment and so created a false impression of simplicity.

c. Chemoreceptors

In insects, as in other terrestrial animals, a distinction is often made between two types of chemoreceptor; the one receptive to vapours at relatively low concentration, normally referred to as olfactory; and the other mediating a response to substances in solution at relatively high concentration, usually called a gustatory, or contact, chemoreceptor. In insects the two types have one thing in common, in that the receptive surfaces of both are bare to the influence of the environment through some kind of aperture in the general cuticular investment. Figure 9.10(a) shows an olfactory sensillum from the antenna of a grasshopper, innervated by a considerable number of bipolar neurones, whose distal processes project up into the perforated cuticular peg to terminate as clusters of microvillar dendrites in the aperture of perforations. In the much investigated contact chemoreceptors, the number of neurones is very much smaller, and their terminal processes extend into the shaft of the chemosensory hair to end just below a terminal pore (see Fig. 9.10(b)).

Early studies of the physiology of chemoreception were based largely on the stereotyped responses which in many species of insect can be elicited by suitable stimulation of chemoreceptors. For instance, if the tarsal sensilla of a hungry blowfly are brought into contact with a solution of sucrose, the proboscis of the fly is usually lowered in preparation for the act of feeding. By determining the threshold for proboscis extension with different materials which evoke a positive response, or alternatively by determining the rejection thresholds for substances which cause rejection when presented together with a substance that would otherwise elicit a positive response, a lot of useful information has been obtained concerning the physiology of the receptor process. It has been shown, for instance, that the rejection threshold for homologous series of alcohols and

Fig. 9.10. Insect chemoreceptors. (a) Section through an olfactory sensillum from the antenna of a grasshopper, showing dendrites (D) from a large number of bipolar neurones (N) enclosed for part of their length in a scolopoid sheath (S) extending into the peg, and terminating at the pegwall perforations. A, axons. The inset shows a section through one of the perforations with dendritic microvillae in the aperture (Dethier, 1963). (b) Section through the tip of a tarsal chemoreceptor of the stable fly, showing the pore (a) at the end of the sensillum, below which the neurone dendrites (b) terminate (drawn from electron micrograph of Hodgson, 1964, courtesy Adams).

glycerols decreases with increasing length of the carbon chain, and that a discontinuity occurs in this relation at the point where oil/water partition coefficients show an abrupt change, suggesting that the effectiveness of compounds in causing rejection is in part associated with lipid solubility.

The main objection to the use of behavioural criteria for investigations of sensory physiology is that what is being tested is not the sensitivity of the sense organ, but the reactivity of the whole of the neuronal network interposed between the sensory and the motor fields. Critical exploration of chemosensory physiology had, therefore, to await the development of electrophysiological techniques sufficiently refined to detect the activity of the sensory neurones themselves. Olfactory receptors have proved rather intractable in this respect,

and most investigators have here had to rely on a study of summed responses in large numbers of antennal receptors to provide a measure of activity. Such "electroantennograms" have been shown to differ in form depending on the nature of the stimulating odour (see Fig. 9.11(a)), particularly characteristic patterns being obtained with the antennae of male silkworms when they are stimulated by the female sex-attractant. The very large discrepancy between the concentration of attractant necessary to elicit a distinctive electrophysiological response and that required to initiate the corresponding behavioural reaction suggests, however, that the approach is too crude to be of use for investigations of the neurophysiological basis of the response.

Electrophysiological investigations of gustatory sense organs have met with considerably more success. Here the number of sense cells involved in a single sensillum is small, and the response of different neurones can often be distinguished on the basis of spike height. By the ingenious use of capillary electrodes placed over the tip of the sensillum, providing at the same time a means of monitoring the electrical potentials developed as a result of stimulation and a means of applying the gustatory stimulus, the appropriate substances being incorporated in the solution of the capillary electrode, it has been possible to investigate the physiology of chemoreception at the level of the sensory neurone. In the labellar chemoreceptors of the blowfly two types of afferent potential, large and small, can be distinguished (Fig. 9.11(b)). These have been shown to be evoked in response to the application of inorganic salts and of sugars respectively, and the frequency of impulse discharge has been found to be linearly related to concentration over the range of 0.03-1.00 M.

The specificity of the response to sugar was investigated by testing a variety of different types of carbohydrate. One of the most important properties of active substances was found to be the presence of an α-D-glucopyranoside linkage, as in sucrose and maltose, a finding which is fully substantiated by behavioural studies. It is postulated that the terminal surface of the carbohydrate receptor incorporates a highly specific receptor site, with which only sugars of the right molecular configuration can react; and that the interaction between receptor surface and stimulating molecule in some way causes a depolarization of the membrane to produce a generator potential, which in turn initiates a discharge of action potentials in the afferent nerve. It has recently proved possible to monitor the graded generator potential itself (see Fig. 9.11(c)), by recording from the fractured base of the chemosensory hair.

A third chemoreceptor has been identified in the labellar hair of the blowfly, responding to the application of pure water by low amplitude impulses detectable through the side wall of the sensillum. The discharge is inhibited by sucrose, and provides a neurophysiological basis for the ability of blowflies to distinguish water from other acceptable substances. It should also be mentioned that a mechanoreceptive neurone is associated with the articulated socket of the chemosensory hair, giving the single sensillum a dual function.

(a)

(b)   (c)

Fig. 9.11. Aspects of the electrophysiology of chemoreception. (a) Electroantennogram from the isolated antenna of a female moth subjected to stimulation by currents of air carrying different vapours. a, unpurified air; b, air plus wintergreen-oil vapour; c, air plus clove-oil vapour; d, unpurified air; e, air plus female sex-attractant of same species; f, air plus sex attractant of different species. Note the absence of response in the female antenna to sex-attractant which in a male antenna would give a response similar to trace c. The duration of air currents is marked by horizontal bars above the EAG (Schneider, 1962). (b) Spike potentials recorded by capillary electrodes on the labellar chemosensory hairs of the blowfly. A, the large fibre response to stimulation with 0.5 M NaCl; B, large and small fibre response to stimulation by a mixture of 0.25 M sucrose and 0.1 M NaCl (Hodgson, 1964). (c) Generator potentials and spike potentials from labellar hair of blowfly. Single stimuli applied to obtain record on the right. The left record made following application of a mixture of salt and sugar, showing partial summation of generator potentials (Hodgson, 1964, courtesy Marita).

d. Humidity Receptors

Brief mention must be made of the ability of many insects to respond to humidity, to water as a vapour rather than as a solvent. The sense organs involved have been identified on behavioural criteria in a number of insects, but nothing is known of the electrophysiological basis of humidity reception. In some cases it has been possible to establish that the response is to evaporating power rather than to water vapour, in others relative humidity appears to be the

effective stimulus. Precisely what it is that mediates afferent discharge is, however, still unknown; it might be water vapour acting as an olfactory stimulus; it might be changes in the physical conformation of receptor structures based on cuticular hygroscopy; or it might be changes in osmotic conditions, or in temperature, associated with the evaporation of water from receptor surfaces. Until electrophysiological methods can be refined to the point where changes in the electrical potential of receptor membranes can be detected, there seems little hope for progress in this field.

e. Temperature Sensitivity

Many insects are known to respond to temperature, and the antennae are often involved in such responses. It is possible that the reaction may be mediated by receptors sensitive to other modalities, whose activity may be modulated by changes in temperature, as the activity of the chemosensory neurones is known to be. The high degree of discrimination shown by certain species makes it likely, however, that specific temperature receptors exist, though their identity has not yet been unequivocally established.

CHAPTER 10

INTEGRATIVE ASPECTS OF NERVOUS FUNCTION

While important advances have been made during recent years in the elucidation of unit function, progress in the field of nervous integration has been slow. One might imagine that insects would constitute particularly favourable material for the investigation of the physiology of integration, in view of the relatively small number of elements which are involved both on the sensory and the motor side. It seems, however, that the advantages conferred by neural parsimony are to a large extent offset by technical difficulties associated with the special architecture of the central nervous system in insects. In the first place, there is no convenient separation of afferent from efferent routes in insect nerves; the nerves are mixed from their point of origin in the central nervous system, carrying both sensory and motor fibres, so that neither in the stimulation of nerves, nor in recording from them, is it possible to distinguish the two parts of the reflex arc. The second difficulty arises from the nature of the neuropile in insects. The large neurone cell bodies, from which intracellular recording would be technically possible, are situated at points remote from the interplay of synaptic effects, and provide correspondingly little evidence of the nature of such effects. To investigate the electrical activity of the neuropile, recourse must be had to extracellular recording, and the results have so far been disappointing; for while it is possible to pick up active units by this means, such units appear to be sparsely distributed, and separated by large tracts of electrically silent tissue. The active units can often be shown to come under the influence of sensory input, as illustrated in Fig. 10.1, where the response of a brain unit to flashes of light is recorded. But the lack of variety and discrimination in the pattern of response of such units has been a cause for concern among investigators. The severe restraint under which the insects have to be placed in order to permit recording may be in some part to blame for the apparent lack of meaningful response, for the fact that, as one research worker puts it, "almost all the recordings can be interpreted as alarm responses dulled by repetition". But apart from this, the prospects for an elucidation of the neurophysiological basis of integration will remain poor so long as the activity of a high proportion of neuropile units defies detection with present instrumenta-

Fig. 10.1. The response of a brain unit to successive presentations of identical flashes of light presented at 5-s intervals. The long latency at "on", together with the pause after the initial spike in the first line, suggests that the "on" response has been suppressed by inhibition. The "off" response shows progressive habituation (Horridge *et al.*, 1965).

tion, since it is presumably the smaller, silent elements that serve as the basis of integrative activity.

One other factor militates against progress in the field of central nervous function, and that is the lack of accurate maps of the general anatomy of the central nervous system in insects; only a few species have been carefully investigated, and these not the ones that are most suitable as experimental material. It would seem that until this deficiency has been made good, and until refinements of neurophysiological technique open the way to investigation of smaller neuropile elements, the finer details of integrative function will remain

beyond our reach. The best that can be done for the present will be to establish the relation between input to the central nervous system and output from it. Both can often be precisely defined in terms of unit activity, and the relation between them can be determined in quantitative terms; how, precisely, this relation is established within the integrative networks of the neuropile will have to remain a problem for the future.

The pattern of input from different types of sense organ has been briefly outlined in the previous chapter; in the first part of this one, a few cases will be discussed where attempts have been made to relate changes in input, as defined in electrophysiological terms, to changes in output, as defined in broader behavioural terms. In the second part the approach will be made from the motor side of the reflex pathway, and the pattern of activity in the final common path, defined in electrophysiological terms, will be related to changes in input which can sometimes be defined in quantitative terms, sometimes only in general terms. In the last part consideration will be given to the effect on output of operative or electrical interference with nerve centres remote from the final common path. Here the nature of the interference can only be defined in gross anatomical terms, and interpretation becomes correspondingly uncertain.

1. The Neurophysiology of Escape Reactions

a. The Startle Response of the Cockroach

One of the most striking characteristics of the central nervous system of insects, as of many other invertebrates, is the so-called giant fibre system. This generally mediates escape reactions of some kind, as in the startle response of the cockroach, where the speed with which the reaction can be performed may have dramatic adaptive significance, making the difference between the life and death of the individual concerned. The response latency of such reactions has in many species been greatly reduced by an increase in the diameter of certain internuncial nerve fibres (see Fig. 10.2(a)), which has resulted in a corresponding increase in the conduction velocity. Because of their large size, the activity of these giant interneurones is readily monitored, and most of the reflex components are thus open to electrophysiological investigation. The startle response of the cockroach has been particularly carefully studied, and details of the reflex pathway between the sensory field, as represented by mechano-receptors on the anal cerci and the motor field, as represented by the musculature of the metathoracic leg, are illustrated in Fig. 10.2(b).

Stimulation of the delicate tactile sensilla by a puff of air leads to a massive discharge of sensory neurones and provides an afferent input to the giant internuncials in the last abdominal ganglion. The size of the resulting excitatory postsynaptic potential developed across the giant fibre membrane, increases with increasing intensity of stimulation, by spatial and temporal summation,

(a)

100 μ

	msec
Latent period of receptor activity	0·5
Conduction time	1·5
Synaptic delay	1·5
Conduction time	2·8
Conduction time	1·5
Neuromuscular transmission	4·0
Contraction latency	4·0
Total	15·8

(b)

Fig. 10.2. Neuromuscular basis of the startle response of the cockroach. (a) Giant fibres in a cross-section of the ventral nerve cord of the cockroach (drawn from photomicrograph in Huber, 1965, from Roeder). (b) Diagram of the neuromuscular elements involved in the startle response of the cockroach, with the duration of unit events listed at the right of the figure. The linear scale has been greatly distorted for ease of representation. c.n., cercal nerve; f.f., fast fibre; g.f., giant fibre; m, muscle; m.e.p., motor end plate; m.c., motor centre; s.n., sensory neurone (from Roeder, 1963).

until it is sufficient to trigger a discharge of action potentials in the giant fibres. The impulses sweep up through a succession of abdominal ganglia to the metathoracic ganglion, where the giant fibres divide to form a tangle of smaller branches embedded in the neuropile. They transmit ultimately to the fast motoneurones of the metathoracic leg, whose discharge causes contraction of the leg muscles, and so initiates the escape reaction.

Most of the unit events in the reflex pathway have been accurately timed, and estimates of duration have been included in Fig. 10.2(b); the sum for known unit elements is about 16 ms. There is a substantial difference between this value and experimentally determined latencies for the reaction as a whole, which average 54 ms, with a minimum of 26 ms. The difference must clearly be attributed to the time taken for transmission through what may be called the "motor centre" of the metathoracic ganglion. What is involved in the motor effect is not simply the contraction of a muscle, but rather the co-ordinated movement of a limb, and the substantial time that intervenes between the arrival of giant fibre impulses and the initiation of activity in the final common paths suggests that a neuronal network of considerable complexity may be interposed at this point. This is borne out by the very labile and unpredictable nature of the response at this level of the reflex arc, which has so far precluded detailed electrophysiological analysis. Transmission through the metathoracic ganglion often appears to block irreversibly under experimental conditions, and under natural conditions it is this point that appears to come under inhibitory influences, which may cause the behaviour pattern to extinguish completely after only a few trials. This is an example of the general phenomenon of habituation, which will be discussed further in Chapter 11.

b. Predator Evasion by Noctuid Moths

The startle response of the cockroach is essentially an all-or-nothing type of response, involving a minimum of discrimination at the level of input; the motor pattern does not appear to be directed in relation to the stimulus, nor guided by its form. Either the sensory discharge is sufficient to initiate activity in the giant fibres or it is not; if it is, then the behaviour pattern may be triggered off, or it may not, depending on the state of the motor centre, which is the point at which discrimination appears to occur. By contrast, the evasive reactions of certain noctuid moths during pursuit by bats is closely related to the nature and the source of stimulation, reflecting a much greater content of information at the level of the sensory input. In this case, however, no giant internuncials are involved, and the input is lost in the silent regions of the neuropile as soon as it enters the central nervous system. The relation between input and output can therefore only be gauged by observation of behavioural performance.

The sense organs involved in predator evasion are the paired tympanic organs, each consisting essentially of a thin membrane stretched on a cuticular frame.

Movements of the membrane induced by airborne vibrations cause a discharge in two sensory neurones associated with the tympanum. The sensory physiology of this "ear" has been investigated in detail by Roeder, whose results show that it is sensitive to a very wide range of frequencies, with a sensitivity maximum at about 60 Kc/s, a frequency which is well represented in the ultrasonic, echo-locating cries of insectivorous bats. The discharge frequency in the tympanic nerve is a linear function of intensity over a range of about 40 decibels above which the receptors saturate.

The sensory basis of predator evasion is thus of the greatest simplicity, but in spite of this the information content of the sensory input is substantial, as illustrated by records of tympanic discharge evoked by the echo-locating cries of a hunting bat. The cries consist of short bursts of high frequency sound produced at a rate of about 10/s during cruising, with the pattern changing to a rising crescendo or "buzz" of pulses delivered at a higher rate, as the bat closes in upon its prey. In Fig. 10.3(a) the discharges of left and right tympanic nerves of a restrained moth are recorded in the presence of a hunting bat. Record A shows the response to an approach by a cruising bat, signalled at first by a slight discharge from only one tympanic membrane, thus providing information concerning the direction of approach. The second cry is picked up by both organs, the higher intensity of discharge indicating that the bat is approaching; information about direction is still available on the basis of differences in discharge frequency. The third response is similar, but in the fourth the pattern of discharge on the two sides is virtually identical, indicating that at this stage the bat is directly overhead. In record B the response to the closing "buzz" of a hunting bat is shown, as registered mainly by one of the tympanic organs, while in record C the "buzz" is registered by both.

These results show the mechanism by which the discharge of tympanic organs provides information concerning details of the behaviour of hunting bats. The discharge frequency gives an indication of distance; changes in frequency over a succession of bursts indicates whether the predator is closing in or moving away; and differences between the discharge from left and right tympanic organs provide information concerning the direction of approach. Precisely how these differences in input affect the pattern of motor output cannot at present be determined; but that they do, in fact, exert an appropriate influence is evidenced by the overt behaviour of moths in response to stimulation by artificial bat cries. Figure 10.3(b) shows the effect of stimulation by short bursts of ultrasonic vibration on the flight path of moths. The source of stimulation, represented by a hatched circle, is mounted on a 14-ft mast, and two types of response are illustrated. One is the response to high intensities of sound (i and ii), which may be either a power dive, or a passive dive with wings closed. Here the direction of movement bears no relation to the source of stimulation, presumably because at high intensities the tympanic discharge saturates, and information about the

Fig. 10.3. Predator evasion of moths. (a) Impulse discharge in right and left tympanic nerves of a moth, in response to the cries of a red bat flying in the field. The slow waves represent depolarizations of the heart, and the large spikes which appear singly without synchrony are discharges from a third receptor associated with the tympanic organ, of unknown function. A, an approaching bat; differential response between right and left is marked at first but disappears as the bat flies overhead; B, a "buzz" registered mainly by one ear; c, a "buzz" registered a few seconds later by both ears (Roeder, 1963). (b) Flight paths of moths in response to an artificial ultrasonic sound pulse sequence. The hatched circle represents the source of stimulation. (i) and (ii), responses to high intensities of stimulation; (iii) and (iv), responses to low intensities of stimulation; dotted arrows mark the moment of stimulation. (Schematized from flash photographs of Roeder, 1963.)

direction of the stimulus source would therefore be lost. At lower intensities of stimulation (iii and iv) it was possible to obtain clear indications of a directional component, with the moths taking a flight path directly away from the source of stimulation.

These experiments show that features of the sensory input are appropriately reflected in the pattern of motor output, even though it has not yet been possible to interpret the different output patterns, or to define the mechanism of integration, in terms of unit activity.

2. Patterns of Activity in Motoneurones

The one element of nervous architecture whose destination can be readily identified, and whose activity can be readily monitored, is the motoneurone, and a great deal of information has accumulated concerning patterns of activity in different types of motoneurone. Two examples have been selected for detailed discussion, to illustrate the sorts of results that have been obtained.

a. The Motor Supply to Spiracular Muscles

The neurophysiological basis of spiracular regulation has been under intensive investigation during recent years, and the results obtained furnish a convenient example of the effect of input, and of endogenous influences, on the pattern of activity in a motor unit. They have been schematized in Fig. 10.4, which will serve as a basis for the discussion that follows.

The motor supply to the closer muscle of a spiracle is shown as comprising two axons in the median nerve of the corresponding thoracic ganglion, the axons dividing immediately after they emerge from the ganglion to supply right and left spiracles. In the absence of ventilation these axons are continually active, firing at slightly different frequencies of about 10-20 impulses/s, so that recordings from the nerve show action potentials coming in and out of phase at regular intervals. This "free-running" pattern of activity gives rise to alternate periods of "fluttering" of the spiracular valve (see Chapter 7) coincident with

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Fig. 10.4. Aspects of the neurophysiology of spiracular regulation. The site of action of various influences is indicated in the sketch, which shows the spiracle with its closer muscle on the left and two thoracic ganglia on the right. (a) The effect of a short burst of carbon dioxide, as marked by the arrows on: - - - - the magnitude of the muscle action potential; —— the tension developed during a twitch contraction; and . . . . the depolarization of the muscle membrane. (b) The relation between the frequency of motor impulses and the concentration of carbon dioxide required to open the spiracle. (c) The relation between impulse frequency and the different phases of the ventilation cycle. (d) Changes in impulse frequency recorded when the perfusion medium is switched from half strength to double strength of physiological saline. (e) The effect of oxygen and of carbon dioxide concentration on the discharge frequency. (f) The effect of temperature on discharge frequency. (g) Spiracular mechanoreceptor. Schematized diagrams from Hoyle, 1960(a) and from Miller 1964a b, 1965 (b-f).

synchronous firing, and of full closure when impulses arrive out of phase. This appears to constitute the background against which the control mechanisms of spiracular regulation operate.

The control mechanisms are of two different kinds, peripheral and central:

(1) the peripheral effects are based on a direct action of carbon dioxide on the closer muscle. The precise nature of this effect has not yet been fully elucidated, but carbon dioxide appears to cause a depolarization of the muscle membrane, which leads to a reduction in the magnitude of end-plate potentials, and a corresponding reduction in the force of contraction (Fig. 10.4(a)); and

(2) the central effects are mediated by changes in the frequency of discharge of the motoneurone, which affect the sensitivity of the closer muscle to the peripheral action of carbon dioxide. The higher the discharge frequency, the greater the concentration of carbon dioxide necessary to produce relaxation (Fig. 10.4(b)). A number of factors have been shown to influence the frequency of discharge of the motoneurone, of which the most important are:

(i) Ventilation. The motoneurones appear to be under the influence of excitatory and inhibitory interneurones, whose activity is linked to the cycle of ventilation, causing an increase in discharge frequency during expiration and an inhibition of discharge during inspiration (Fig. 10.4(c)). The details vary considerably from species to species, but the over-all effect is to synchronize the opening and closing of spiracles with the phases of ventilation, as described in Chapter 7. At the initiation of flight the motoneurone discharge is completely inhibited and spiracles open fully.

(ii) Water Balance. The state of water reserves has been shown to have a profound influence on the frequency of discharge in spiracular motoneurones, an effect which is thought to be mediated by changes in haemolymph concentration. The effect can be mimicked by perfusion of the metathoracic ganglion with saline solutions of different concentration, as illustrated in Fig. 10.4(d). Dilute solutions are associated with a low discharge frequency, while concentrated solutions produce high frequencies of discharge. On this basis, an insect whose haemolymph has been concentrated by dehydration would be expected to exercise stringent spiracular control, in agreement with the low rates of water loss which characterize insects in this state (see Chapter 7).

(iii) The Concentration of Respiratory Gases. If insects are exposed to gas mixtures containing subnormal concentrations of oxygen, there is a marked decrease in impulse frequency, which, in the well-hydrated insect, reaches zero, and thus ensures full opening of the spiracles, at a level of about 2% oxygen. Conversely, there is a decrease in discharge frequency as the carbon dioxide concentration increases above 10% (Fig. 10.4(e)).

(iv) Temperature. An increase in temperature produces a marked increase in discharge frequency over the range from $10°$ to $35°$, as illustrated in Fig. 10.4(f).

(v) Mechanical Stimulation. Mechanical stimuli applied to the region of the spiracular filter cause a discharge in associated mechanoreceptors (Fig. 10.4(g)). This produces an excitatory input to the motoneurone, and causes a burst of high frequency discharge to the closer muscle, and rapid closure of the spiracle. This presumably constitutes a mechanism by which the entry of dust particles or parasites is prevented.

Apart from the reflex response to mechanical stimulation, the precise nature of the effects described has not yet been established. Some of them are capable of being mediated by the isolated metathoracic ganglion, and could be ascribed to a direct effect of the factors concerned on the motoneurones themselves; on the other hand, it is possible that they may be based on afferent inputs originating in specific sense organs associated with the ganglion. The nature of ventilation control is also obscure; effects might be associated with the activity of stretch receptors monitoring the stresses developed during ventilation, or they could represent a collateral output from the motor centres controlling ventilation. Whatever the detailed interpretation, the results which have been obtained furnish a good example of the way in which the activity of motor units may be regulated by a variety of influences to produce a response that is homeostatic in the context of respiratory exchange and of water balance. Spiracular regulation appears to be governed predominantly by respiratory needs; it is carefully integrated with the phases of ventilation, and the peripheral carbon dioxide effect ensures that the needs of respiratory exchange are met according to the demands of the moment. The threshold of the carbon dioxide effect, however, is adjusted in the interests of water conservation, so that the frequency with which the spiracles open is reduced under conditions of desiccation. The temperature effect is thought to be related to the need for maintaining high thoracic temperatures for peak flight performance, but it would serve also to reduce losses of water that would otherwise occur at high rates from heated surfaces of the tracheal system immediately following flight.

b. The Coxal Adductor

A motor unit which shows interesting effects of a kind rather different from those described above is that which supplies the coxal adductor muscle of the jumping leg of locusts. This unit is concerned with the maintenance of posture, and it shows a regular tonic discharge in accord with this function. It has been shown that the frequency of the resting discharge is susceptible to changes of a relatively long-term kind, as the result of afferent inputs which are specifically related to its own output. If the frequency of motor discharge is monitored continuously, and if afferent axons of the tibial nerve are stimulated with a single shock every time the frequency of firing falls below a specific level, an increase in the mean discharge frequency is induced. By progressively raising the "demand" level, the mean discharge frequency can be more than doubled, as

illustrated in Fig. 10.5, and the effect may persist for several hours after it has been produced.

Fig. 10.5. Results of an experiment in which a shock was given to the tibia of a locust each time the mean discharge frequency over a 10-s period in the nerve of the right metathoracic anterior coxal adductor fell below a prescribed arbitrary "demand" level, indicated by the broken line. Moments of stimulation are indicated by arrows below the demand line. The dots show the mean frequency over a 10-s period, whilst the bars show the mean frequency over 100-s periods, to indicate the general trend more clearly. Note that after a few stimuli have been received the mean frequency rises. At very high demand levels an inhibitory effect appears to supervene (at arrows marked 1). (Hoyle, 1965.)

These results provide an adequate basis for interpretation of the original demonstration by Horridge (1962) of "learning" in a headless insect. This involved a preparation set up in such a way that the lowering of the metathoracic leg of a decapitated cockroach closed a stimulus circuit, and so delivered an electric shock to the lowered leg. It was found that after a relatively short period of time (10 min or so) the preparation "learnt" to avoid the electric shocks, by keeping the leg in a raised position. When this effect is divorced from the rather bizarre experimental situation used for its demonstration, and interpreted in the light of the experiments with locusts, it is clear that it may constitute a phenomenon of quite general importance. The results suggest that afferent inputs which follow systematically on significant trends in the motor output would work to produce a reversal of such trends. Mechanisms of this kind would constitute a satisfactory basis for the reflex adjustment of posture, for example, and as such would be of undoubted adaptive significance. Interest would centre on the precise neurophysiological basis of the underlying plastic change in nervous function, but it is unfortunately only too probable that the change occurs among the unseen activities of the smaller neuropile elements, and that it would therefore lie outside the range of experimental attack for the time being.

3. Central Inhibition

In an earlier part of this chapter the concept of motor centres was introduced, envisaged as neuronal networks capable of mediating an output, appropriately patterned in time and space, to a complex field. The anatomical basis of such hypothetical centres remains obscure, but the effects of operative procedures to be described in the present section add some support to the notion of their existence, whatever their precise nature. The free-running discharge of the single motor unit described above could be considered as representing one small fraction of the output from such segmental motor centres.

The most convincing demonstration of the activity of segmental motor centres comes from work on the reproductive behaviour of the praying mantis. One of the characteristic elements of the reproductive behaviour of the male of this species is an S-shaped bending of the abdomen, which serves to direct the terminal segments forward, and so permit the ovipositor of the female to be probed by the genitalia of the male. It has been shown that this motor pattern can be released by decapitation, or by transection of the ventral nerve cord at any point, and that it is the suboesophageal ganglion which, under normal circumstances, exercises an inhibitory influence over the lower segmental centres. It would seem that the sexual movements which occur in the decapitated mantis are the expression of appropriately patterned outputs from the abdominal ganglia, which might be produced endogenously, and without reference to sensory feedback. As mentioned in the introduction to this chapter, it is not possible to test this suggestion directly, by de-afferentation of the active centres, because of the mixed nature of insect nerves; but the concept of an endogenous origin for the patterned activity receives some support from the records illustrated in Fig. 10.6(a). These show the activity of motor units in a nerve passing from the last abdominal ganglion to the phallic apparatus, severed at a point distal to the electrodes, and with all other nerves to the ganglion cut to prevent input from segmental sense organs; the only connection between the ganglion and the rest of the nervous system is through the ventral nerve cord. The first part of the record shows the low level of discharge which occurs when the connection with the central nervous system, in particular with the suboesophageal ganglion, is intact. The second record shows an increase in activity 3 min after transection of the nerve cord, building up to the massive discharge of the last record, made after 7 min. If the records obtained over fairly long periods are examined, discharge frequencies in different motor units can be seen to wax and wane in a regular pattern, and it seems reasonable to suppose that these fluctuations represent the neurophysiological counterpart of the rhythmic copulatory movements which would have taken place had the nerves to the phallic musculature not been cut. If this interpretation is correct, it

Fig. 10.6. The apparent release of motor centres from the inhibitory influences of higher centres. (a) Pattern of motor nerve impulses in the phallic nerve of the mantis. A, before connectives between the last abdominal ganglion and the rest of the nervous system have been severed; B, 3 min after transection of the nerve cord; C, 7 min after transection of the nerve cord. Time trace 100 cyc/s (Roeder, 1963). (b) Spontaneous efferent nerve activity in the isolated thoracic nerve cord of the cockroach. TH3, metathoracic ganglion with

demonstrates that the motor pattern is independent of sensory input, and lends support to the view of an endogenous origin.

In the praying mantis the release of sexual activity following decapitation is particularly strikingly manifested, and in view of the cannibalistic tendencies of the female mantis during courtship, which sometimes lead to decapitation of the male, it may well have considerable adaptive value. A similar phenomenon has, however, been demonstrated in the cockroach, where transection of the ventral nerve cord leads to the appearance of rhythmic bursts of motor impulses in nerves of the terminal ganglion. Here the corresponding motor activity is too attenuated in form to promote effective coupling, as it often does in the headless mantis, but there can be little doubt that the principle is the same, namely that of a segmental centre capable of a patterned endogenous output, but held under inhibition by higher centres. Nor is the phenomenon restricted to sexual patterns mediated by abdominal ganglia; Fig. 10.6(b) shows spontaneous activity in one of the nerves associated with the metathoracic ganglion of the cockroach, innervating coxal and other muscles of the metathoracic leg. In the presence of the head and the de-afferentated thoracic ganglia there is a low level of activity, but after connectives to the brain and suboesophageal ganglion have been cut there is a progressive increase in discharge rate, which reaches massive proportions after about 30 min. The change involves not only an increase in the firing frequency of previously active units, but also the bringing into operation of new units.

Inhibition of the activity of segmental motor centres is exemplified also in a grooming reflex of the locust, which has been investigated by Rowell (1965). The reflex can be elicited in a locust, restrained ventral surface uppermost, by a touch to the tactile receptors of the sternal region of the prothoracic segment. This elicits movements of the front legs directed towards the point of stimulation; both afferent and efferent pathways are confined to nerves of the prothoracic ganglion. In the intact preparation responsiveness is extremely low, and the reflex can rarely be evoked, but by a variety of operative procedures responsiveness can be greatly increased. If the nerve cord is severed anterior to the prothoracic ganglion, consistent results can be obtained, with the preparation responding about once in 10 trials. If, now, the input from lower parts of the central nervous system is progressively reduced, by de-afferentation of ganglia, or by severing the ventral nerve cord at different levels, there is a corresponding increase in responsiveness. The increase that results from isolating the prothorax from the abdominal chain is slight but significant; de-afferentation

connectives and lateral nerves; a, anterior; b, posterior; 4, lateral nerve innervating mainly coxal muscles and tergal and pleural depressors of the third leg. A, motor activity recorded from nerve 4 in a preparation that includes head and thoracic ganglia, without sensory input; B, the same 1 min after cutting the brain/suboesophageal connections; and C, 30 min later. Calibration 0.5 mV; time marker 50 cyc/s (Huber, 1965).

of the metathoracic ganglion now causes a marked increase in responsiveness, with positive reactions occurring approximately three times in every 10 trials. If the nerve cord is severed between meta- and meso-thoracic ganglia, the response rate rises to 8 in 10, while de-afferentation of the mesothoracic ganglion produces a preparation which responds all but invariably. Here appears to be another example of the release of a motor centre from inhibition, this time exerted by both higher and lower centres; and in this case the reflex appears to be steered in relation to the afferent input in such a way as to produce an appropriately orientated response, a condition which would be expected to apply to the operation of most motor centres under normal circumstances. One would imagine that the endogenous motor output, arising as the result of activity in a particular neuronal network, would produce a general pattern of movement whose finer details would be capable of adjustment on the basis of afferent input and feedback.

Experiments of the type described cannot provide more than an indication concerning the general principles of neuronal architecture. The operative procedures are too drastic to enable particular centres to be identified, since they usually involve the severing of an unknown number of fibre tracts of unknown origin and destination. A more promising approach to the elucidation of the details of nervous organization has been adopted by investigators using electrical stimulation of nerve centres in unrestrained animals, through chronically implanted electrodes. A motor pattern which has proved particularly amenable to this type of investigation is that involved in the sound production of crickets, based on the activity of stridulatory muscles innervated from the second thoracic ganglion. Three different types of song can be distinguished on the basis of differences in the pattern of sound—a calling song, a courtship song and an aggressive song. The performance of the corresponding motor patterns appears to be dependent on the activity of brain centres, and singing fails completely if connectives are severed between the brain and the thoracic ganglion. In this case there is clearly no question of a release of lower motor centres from the inhibitory influence of higher; on the contrary, there would appear to be an excitatory input from the higher levels.

The so-called mushroom bodies, or corpora pedunculata, of the supra-oesophageal ganglion seem to be closely involved with sound production in particular. They are situated one on each side of the protocerebrum, and consist of a massive neuropile receiving input from cephalic sense organs, and containing interneurones whose axons and collaterals appear to be restricted to the mushroom bodies. By electrical stimulation of certain parts of the neuropile, and of its associated fibre tracts, it is possible to evoke the calling and the courtship song (see Fig. 10.7); in other parts there may be an inhibition of singing during stimulation, while stimulation of the adjacent "central body" causes the production of atypical sounds. The performance of the different types of song is

Fig. 10.7. Song patterns of male crickets and responses during stimulation of various parts of the brain. (a) Inhibition of calling by stimulation of the calyx of the mushroom bodies. (b) The calling song elicited by stimulation of the α-lobe. (c) The aggressive song elicited by stimulation in the tractus olfactorio globularis. (d) Atypical sounds elicited by stimulation of the central body neuropile. (e) Diagram of the cricket brain with the mushroom bodies and central body indicated by dotted lines and showing the distribution of points of stimulation. White squares, points of inhibition; black triangles, points at which calling is elicited; half-filled circles, points at which aggressive calling and associated behaviour is elicited; black circles, points at which atypical sounds are elicited; c, calyx; pe, peduncle; ca, α-lobe; pc, protocerebral bridge; cc, central body; PC, protocerebrum; TC, tritocerebrum (Huber, 1965).

accompanied by the postures and associated movements characteristic of the natural performance of the behaviour pattern, and the results fully confirm the long-accepted view that the mushroom bodies constitute important centres regulating the performance of complex patterns of behaviour.

This approach provides a means of identifying the nervous centres responsible for the regulation of motor activities much more accurately than can be done on the basis of ablation experiments. Unfortunately it represents no more than a first step toward an elucidation of details of higher integrative function; for substantial progress in this field a thorough knowledge of the distribution of dendritic fields and fibre tracts will be required, and technical advances must be

made in relation to pin-point stimulation and recording. Here, as at the lower levels of nervous integration, the promise which is afforded by insects for the elucidation of the neuronal basis of behaviour, by economy of nervous elements, is counterbalanced by the technical difficulties posed by the nature of the insect neuropile, both for the neurophysiologist and for the anatomist.

BEHAVIOUR

The study of insect behaviour has a history that stretches back over the centuries, and an enormous volume of literature has accumulated on the subject. Systematic investigation may be said to have started with the early naturalists, among whom the name of J. H. Fabre deserves special mention, but the investigation of insect behaviour was given a new direction and a fresh impetus during early decades of this century, under the influence of the simple mechanistic views which then prevailed. Insects, like other animals, were considered essentially as complicated machines, constructed to react to external stimuli in fairly simple and definable ways, and they were found to constitute particularly favourable material for experimental investigation of the relations between stimulus and response. Under the influence of this concept insect behaviourists proceeded to establish, in quantitative terms, the reactions of different species to a variety of physical factors, in a phase of investigation which may be said to have culminated with the publication in 1940 of "The Orientation of Animals" by G. Fraenkel and D. L. Gunn. By this time it was becoming clear that the behaviour of animals was not, in fact, capable of being convincingly interpreted in such simple terms, and that progress would have to depend on the formulation of a more satisfactory conceptual framework of interpretation. This was provided in the first place by results obtained from the study of behaviour in higher animals. These results suggested that what might be considered as the unit of behaviour was not so much a reflex as a movement, or "fixed motor pattern"; a movement which did not necessarily require an external stimulus for its evocation. They suggested, in other words, that what is of central importance in the investigation of behaviour is what movements an animal performs rather than the place to which such movements direct it; what it does rather than where it goes. The fixed motor patterns which, on this view, serve as the basis of behaviour may involve no more than the contraction of a single muscle, but more often they are constituted by a temporal and spatial pattern of activity in one or more motor fields giving rise, for instance, to the complex movements involved in walking or flying. The neurophysiological basis of these motor patterns has not yet been uncovered, but they may be considered

to arise from activity in neuronal networks whose basic structure is geno-
typically determined, corresponding to what in the previous chapter was referred
to as a motor centre. The corresponding movements may thus be considered as
inherited species characteristics in exactly the same sense as an anatomical
feature, such as the shape of a wing or the structure of mouthparts, is
characteristic of a species. This does not mean that the performance of the
movement is an invariable unit of behaviour, but simply that the animal
possesses the inbuilt potentiality for performing this type of movement;
precisely how the movement is made will depend not only on the structure of
the neuronal network, but also on the pattern of activity in it, and this in turn
will be a function of input from other parts of the nervous system.

If this "ethological" approach to behaviour, with which the names of
scientists like Lorenz and Tinbergen are particularly closely associated, can be
accepted as the most fruitful for the interpretation of insect behaviour, then the
first task that faces the student of insect behaviour is to analyse the complex
movements made by insects in terms of their constituent motor patterns, with
special reference to the functional significance of these patterns. Secondly it
would be necessary to consider the evocation of motor patterns in terms of
endogenous and exogenous influences; and to determine the extent to which,
and the mechanisms by which, the movements are steered in relation to features
of the environment, acting through the afferent system. Finally, consideration
would have to be given to possible modifications of the motor performance, or
of its afferent basis, associated with previous experience, in other words to the
existence of learning in insects. The account which follows has been split into
four main sections corresponding to these four aspects of behaviour: the motor
pattern, the evocation of motor patterns, the steering of motor patterns, and
learning.

1. The Fixed Motor Patterns of Insects

The morphological diversity of insects is rivalled, as one might expect it
would be, by the multiplicity of motor patterns manifested by different
members of the group, and detailed discussion would be beyond the scope of the
present volume. They range from simple movements like the "wing-waving" of

Fig. 11.1. Some elements of behaviour. (a) The postures of an ant worker cleaning itself.
A, cleaning right antenna and left foreleg; B, cleaning other legs; C, cleaning the posterior
part of the abdomen (Wallis, 1962). (b) Activity of the mealworm beetle at different
humidities; each triangle represents the proportion of animals active 15 min after induced
activation of all animals; the mean of each group is shown by the large circle (Gunn and
Pielou, 1940). (c) Examples of paths taken by the mealworm beetle in a gradient of
humidity with 97-100% relative humidity on the left and 94-97% relative humidity on the
right; black points show where the animals came to rest. The tracks give some indication
that random turning movements tend to keep the animals on the drier (right-hand) side.
(Gunn and Pielou, 1940.)

(a)

(b)

(c)

male fruitflies during courtship; through various grooming movements (see Fig. 11.1(a)), which may differ greatly in point of detail between species; to the co-ordinated sexual patterns of copulation, which have already been outlined in the case of the praying mantis; and from here to the more complicated movements involved in locomotion, whether walking or flying; and finally to the intricate patterns of motor activity operative during the performance of such special activities as the spinning of cocoons, the excavation of burrows or the construction of special nests.

It is clear from the examples given that specific motor pathways may be common to many different motor patterns; the movement of wings is involved both in courtship display and in flying; of legs in grooming and nest-building, as well as in walking. It is therefore necessary to conceive of the corresponding neuronal networks as showing extensive overlap, with elements of one pattern capable of being manifested as one of the components of another. Such an arrangement would ensure that different patterns could be placed at the service of a variety of different functions; walking and flying might in one instance be at the service of mating, at another at the service of nest-building or feeding; and each activity would be considered as operating within the context of broader categories of behaviour, e.g. reproductive, with the activity capable of being evoked from correspondingly diverse sources, as will be discussed below.

It should be emphasized that the interpretation here presented, of behaviour in terms of neurophysiology, of motor patterns as the expression of activity in complex neuronal networks, is quite hypothetical, and goes far beyond the available experimental evidence. It is adopted simply as an aid to a coherent presentation of the subject, and to emphasize the close relation that exists between the subject matter of this chapter and that of earlier ones, whatever the precise nature of that relation may eventually turn out to be.

One of the most characteristic features of the fixed motor pattern is the variation in intensity with which the movement may be performed, ranging from the barely recognizable "intention movement" to the full and vigorous performance. These differences presumably reflect differences in the level of excitation of the corresponding neuronal networks, based on variations in endogenous or exogenous input. In cases where locomotor patterns are involved, and where general physical features of the environment form an important source of input, we have an example of what, on the basis of earlier behavioural investigations, was defined as a "kinetic" mechanism. If, for instance, the linear velocity, or the frequency, of locomotion is affected by humidity (see Fig. 11.1(b)) the insect would be said to exhibit an orthokinetic response to humidity; if the angular velocity of locomotion is affected, the response would be of the klinokinetic type (Fig. 11.1(c)). Such responses have been demonstrated in a variety of insects, particularly in relation to temperature and humidity; they are of considerable biological importance, because they enable

the insect to make an appropriate response to these important physical features, under conditions where an actual orientation to the stimulus might be impossible, because of the attenuation of its directional component. If, for instance, the gradient between humid and dry regions of the environment is so shallow as to preclude participation of a tactic steering mechanism (see below), the fact that the insect moves faster, or more frequently, in dry air than in humid air means that it will spend proportionately less time in dry regions, and aggregations will therefore occur in humid regions, which may be more favourable for survival. Where angular velocity is involved, similar aggregations may result, although the klinokinesis of insects requires a more searching experimental analysis than it has yet received for the unequivocal substantiation of its mechanism. It is significant, however, that kinetic mechanisms have developed in relation to the diffuse stimuli (temperature, humidity and smell), where the directional component is often too weakly developed for direct orientation.

2. The Evocation of Motor Patterns

Motor patterns differ greatly not only in their nature, but also in the type of situation with which they are associated. Some, like walking or flying, may be said to be of a general type, serving many different types of activity, and often appearing spontaneously, without obvious reference to changes in the stimulus situation. Others are highly specific, associated with particular functions such as reproduction, and only elicited in the presence of a particular stimulus, as for instance an object resembling a member of the opposite sex. It was in relation to these more specific types of motor pattern that the concept of the "innate releasing mechanism" was formulated, envisaged as the afferent analogue of the fixed motor pattern. The innate releasing mechanism can be considered as a sensory mechanism ensuring that the appearance of a particular configuration of sensory stimulation, the appearance of some definable proportion of the sum total of stimuli arising from the presence, for example, of a member of the opposite sex, leads to an input to the neuronal networks underlying a particular motor activity, say "wing-waving", and tending to its activation. The existence of this sort of coupling between innate releaser and fixed motor pattern, so that the presentation of a particular stimulus leads to the evocation of a particular activity, seems at first sight to accord well with a simple reflex interpretation, with behaviour seen as a sequence of motor activities induced by a corresponding sequence of specific stimulus situations. Elements of the reproductive behaviour of the digger wasp, for example, would be capable of interpretation in these terms. Care of the brood in this species involves a sequence of activities which include the digging of a nest; the hunting for caterpillars to provision the nest; oviposition; the bringing of more caterpillars and temporary closing of the

nest, followed by a final closing when the nest has been fully provisioned. A sequence of complex motor activities is clearly involved in the total behaviour pattern, but certain fragments of the sequence could be interpreted in terms of reflex theory. The sight of the nest, for instance, would release the dropping of the caterpillar and scraping; the half-open nest would release digging; the open nest would release turning around, and the sight of the caterpillar would release the activity of pulling the caterpillar into the nest. A careful study of this behaviour pattern has made it clear, however, that motor activity is not evoked in any such simple way. To the digger wasp, the stimulus of a caterpillar may elicit quite different elements of behaviour depending on the context in which the stimulus is set, and on what may be termed the "motivation" of the wasp, which presumably reflects the distribution of excitation between different neuronal networks. If the wasp is hunting, the caterpillar is caught, stung and carried to the nest; but if a caterpillar lies close to the nest when the wasp is filling the entrance it may be used simply as filling material; or if a caterpillar is placed in the nest shaft when the wasp is digging out the nest, it will bring the caterpillar away, exactly as it would any other obstacle, such as a piece of wood. The same object thus releases quite different responses, depending on the state of the animal.

Locomotion and courtship movements, the general and the specific motor patterns, should not be considered as distinct in anything but a relative sense, representing perhaps the opposite ends of a continuous spectrum. The performance of specific patterns is usually triggered by the appropriate stimulus, but by no means invariably. In many cases they can be elicited by stimuli which bear no more than a faint resemblance to the normal, and sometimes they occur in the absence of any apparent change in the stimulus situation, as if spontaneously. Presumably the input from non-specific sources, endogenous and exogenous, has sufficed to raise the excitation of the motor centre to the point of discharge in the final common paths. Conversely, the general motor patterns may be elicited in response to specific stimuli, as walking may be evoked by the stimulus of a member of the opposite sex, as a component of courtship.

The performance of a given motor activity, the discharge from a given motor centre, and the intensity of that discharge, may then be taken as a reflection of the level of excitation in that centre, and this in turn will be a function of the level of input, inhibitory as well as excitatory, to that centre from exogenous and endogenous sources. A balance could be envisaged as existing between these two elements of input such that deficiencies in the exogenous component, inadequacies or absence of the releasing stimulus, may be compensated by high levels of endogenous excitation, while lack of endogenous motivation may necessitate the presentation of "super-normal" stimuli for elicitation of the particular activity.

a. Endogenous Input

The nature of endogenous effects can seldom be precisely defined in the present state of knowledge, and the possibility must be considered that several kinds of influence could be exerted on the excitatory state of a motor centre. There would certainly be effects associated with input from other centres, but in addition there might be direct effects of the chemical environment on the cells of the motor centre. Both could, in general terms, be described as reflecting the activation of the corresponding category of behaviour. For instance, onset of the reproductive phase of the life history would be associated with a variety of physiological and neurophysiological events which could potentially be effective in determining the level of excitation in a specific motor centre. The hormones regulating reproduction might exert a direct action on the membrane potential of particular types of neurone, and indications of such an effect have been obtained by experiments similar to the ones described in the previous chapter; the level of motor activity recorded from the nerves of the last abdominal ganglion of the mantis increases greatly during perfusion of the ganglion with extracts of the corpora cardiaca, in a way that mirrors the removal of inhibitory influences by cord transection. Similar direct effects may be involved in the changes of spinning behaviour which characterizes the wax moth at different stages of metamorphosis; it has been shown that implantation of corpora allata in last instar larvae induces the spinning of a larval cocoon instead of the pupal cocoon which would normally have been produced; and conversely, extirpation of corpora allata in early instars induces the formation of precocious pupal cocoons.

It should be mentioned that the regulation by hormones of different types of motor pattern is often rhythmical, so that the performance of the activity tends to occur at certain points in the 24-hr cycle. Where the motor pattern is locomotion, one gets the familiar diurnal rhythms of activity (see Fig. 11.2); where they involve movements associated with emergence of adults from pupae, one gets the eclosion rhythms, which have been demonstrated in a variety of species. Under normal circumstances the rhythmic endogenous influences appear to be set with reference to the rhythmicity of physical features of the environment, usually light or temperature, and diurnal changes in these tend to reinforce the endogenous rhythm; but the rhythmic activity persists for some time under constant physical conditions, although in the absence of reinforcement it tends to diminish in the magnitude of its manifestation, and to drift steadily out of phase with the 24-hr cycle.

The hormonal control of such diurnal activity rhythms has been convincingly demonstrated in the cockroach. When insects are maintained for a long time under constant conditions the rhythm eventually disappears; rhythmical activity can be re-established in such arhythmic individuals by joining them in parabiosis

Fig. 11.2. Diurnal activity rhythms. (a) Activity of the cockroach in alternating light and darkness when food is obtained only in the light periods (shown between dotted lines). (b) Activity after three weeks of such conditions; during the second and third day food was not supplied, and the small activity peak normally associated with feeding is absent. (c) Activity after food has been introduced as usual for five days and the animals then starved in continuous light. Note that the rhythm continues for a few days, but becomes progressively less marked. (Harker, 1956.)

with another insect whose rhythm is still manifest, that is, by establishing continuity between the body fluids of the two insects. It appears that the hormonal secretions of the rhythmic individual, passing into the haemolymph of the arhythmic individual, are capable of inducing a rhythm in the second insect.

Changes in the pattern of metabolism associated with the growth of oöcytes in sexually mature females might constitute another type of endogenous influence. They could be associated with changes in haemolymph composition of a kind that might directly affect membrane polarization. In addition to such a hypothetical effect, and probably more importantly, the onset of reproductive

activity would be associated with more specific nervous influences from other parts of the nervous system. The distinction between endogenous and exogenous factors would here tend to become blurred, because while some of the effects could be claimed as truly endogenous, those for instance which would result from changes in the level of discharge of stretch receptors associated with distension of the abdomen in pregnant females, others could be considered so only in the sense that they would be secondarily relayed from higher centres, rather than resulting from primary afference. The assumption of the reproductive phase would, in most insects, involve the activation of general behaviour patterns which in turn would occasion changes in afferent input. Early stages might be characterized by some form of appetitive behaviour, based on an activation of locomotor centres, leading possibly to quite specific changes in environment, hence of afferent input and of activity in corresponding neuronal networks, which in turn could exert an influence on the levels of excitation of more specific motor centres, destined for activation at a later stage in the sequence of reproductive behaviour.

At this point discussion becomes so general as to verge on the meaningless, nor would much be gained by an attempt to substantiate the occurrence of such effects with reference to specific examples. The case of a more specific and directly exerted endogenous effect should, however, be mentioned. This is the inhibitory input to a given centre which appears to be associated with the discharge of that centre. It is this phenomenon of negative feedback that forms the basis of the concept of the "consummatory act", the idea that the "goal" of a particular activity is not the attainment of some objective, but the performance of an act. The neurophysiological basis of this feedback inhibition has not yet been elucidated in insects, but it has been investigated extensively at the level of behaviour. It is usually found that when a given motor pattern has been elicited by the presentation of the appropriate stimulus situation, that same situation will fail to re-elicit the pattern if it is presented again, or will do so in a very attenuated form, despite the fact that care has been taken to prevent the achievement of the "goal" of the behaviour (e.g. feeding or copulation), which could markedly affect the general physiological state of the insect. The use of models have proved useful as a tool of investigation in this context. The sexual flight of grayling butterflies, for instance, can be elicited by female dummies. When such dummies are presented in succession, there is a decrease in the proportion of males that respond (see Fig. 11.3(a)) and a decrease in the intensity of their sexual flight. Similarly, the courtship behaviour of the parasitic wasp *Mormoniella* wanes rapidly if the males are presented with a succession of non-receptive females, which could be considered as analogous to artificial models. Not only do the models fail to elicit the courtship in a high proportion of trials, but when courtship does take place its intensity, as gauged by a variety of criteria, falls off sharply (Fig. 11.3(b)). There is no question that the

Fig. 11.3. The effect of repetitive stimulation on the performance of a fixed motor pattern. (a) The proportion of male grayling butterflies responding by sexual flight to successive presentation, at about 1½-min intervals, of a female dummy (Tinbergen *et al.*, 1942). (b) The number of "head series", a repetitive component of male courtship in the wasp *Mormoniella*, elicited in successive courtships with receptive females (curve i) and non-receptive females (curve ii), with 30-s intervals between presentation of the stimuli. (Redrawn from Barrass, 1961.)

extinction of the response can be attributed to neuromuscular fatigue, since other activities can be elicited at full intensity; it appears to be due to some kind of negative feedback associated with the performance of the behaviour pattern itself.

Under normal conditions the performance of a particular pattern of activity would be associated with the attainment of a "goal", with the fulfilment of a "purpose" as judged in anthropomorphic terms. The courtship of *Mormoniella* would lead to mating, the lowering of the proboscis of the blowfly in response to stimulation of tarsal chemoreceptors would lead to the intake of food, and so on. The corresponding changes in physiological state greatly affect the rate at which the behaviour patterns extinguish on repetitive stimulation. In *Mormoniella* copulation actually seems to have an excitatory effect on repetitive performance, so that when the males are presented with a succession of receptive females the complete behaviour pattern may be repetitively performed, and there is little waning in the intensity of the performance (see Fig. 11.3(b)). The feeding response of blowflies, on the other hand, elicited by contact of tarsal chemoreceptors with sucrose solution, extinguishes very slowly on repetitive presentation of the stimulus if the act of feeding is prevented. If, however, the fly is permitted to ingest a quantity of solution, the threshold for a positive

response increases greatly, falling gradually during subsequent starvation to the original low values, as shown in Fig. 11.4. Here there appears to be little direct negative feedback from the performance of the motor activity, but changes in afference associated with the ingestion of food have an inhibitory effect on the performance of the motor pattern. This effect appears to be mediated by impulses from stretch receptors which monitor crop distension, passing via the

Fig. 11.4. Changes in sugar thresholds following ingestion of 2.0 M glucose; the full line gives the threshold for glucose, the dotted line the threshold for another stimulating sugar, fucose. (Evans and Dethier, 1957/8.)

stomatogastric system to the brain. Transection of the recurrent nerve prevents this indirect negative feedback, and the fly will continue to feed until it dies by distension.

The fixed motor patterns are thus susceptible to a variety of endogenous influences, both excitatory and inhibitory, the sum total of which determine whether or not the motor pattern will be performed, or the intensity with which it will be performed, under a particular set of external circumstances; and different types of motor pattern differ enormously in the degree of their susceptibility to such influences. Neither the neurophysiological basis of these effects, nor their site of action, have yet been elucidated.

b. Exogenous Input

(i) The Innate Releasing Mechanism. The nature of the effective releasing stimulus has been established for a great many motor patterns in a wide variety of insects, and a number of examples have already been given. They range from single component situations of great simplicity, as in the proboscis extension of the blowfly, where stimulation of a single labellar hair with a sucrose solution may be sufficient to initiate the complex motor response, and where only a single modality of stimulation may be involved. With other motor patterns a

number of different sensory modalities are involved simultaneously or successively, but the whole may still be at a relatively simple level, as in the reactions to prey of the water beetle, where water-borne vibrations cause release of the motor pattern, which in its final stages is dependent on visual stimulation of a rather unspecific kind. Even here the level of complexity is very considerable when one considers the enormous number of mechanoreceptive and visual sense organs that are involved in the total release. Releasing situations usually reach quite another order of complexity with motor patterns that mediate interspecific or intraspecific interaction, where the total stimulus situation may involve a number of different modalities of sensory stimulation— visual, olfactory, gustatory, mechanical—and where some of its components may in themselves be extremely complex. The sum total of stimuli that emanate, for example, from a female praying mantis, to impinge on mechanoreceptive, chemoreceptive and visual sense organs of the male during copulation would clearly be immense. It appears, however, that the releasing mechanism operates not on the basis of the total stimulus situation, but on what may be considered as an abstract of it, involving emphasis on certain of its elements while others appear to be ignored. For this reason it has proved possible to use appropriate models to release particular motor patterns, and by the use of such models to dissect the innate releasing mechanism into its constituent parts. An example of this approach has already been mentioned in connection with the sexual flight of grayling butterflies, which can be elicited with female dummies. By altering the properties of such models it has been possible to identify the major components of the releasing mechanism (see Fig. 11.5). The experiments indicate that shape is not an important element of the releasing mechanism, since models whose outline give a fairly realistic impression of a butterfly are no more effective in eliciting a response than circles or even rectangles, provided the disproportion between length and breadth is not too great; nor does colour seem to be of much importance in the context of sexual pursuit, although it constitutes a very important element of the releasing situation for another behaviour pattern, that of feeding. The factors which were found to be of special relevance to the release of sexual pursuit were reflectivity, size and nearness, and the type of movement with which the presentation of the model was associated, whether it was moved smoothly across the visual field of the male, or in an irregular way. The optimal model was a relatively large object, as dark as possible, as near as possible, and moved in the typical fluttering way of a butterfly's flight.

Experiments of this kind provide a convincing indication that certain elements of the total stimulus situation have been endowed with special significance for the release of a given motor pattern. Such discrimination is presumably based on special configurations of afferent and interneuronal pathways, such that the pattern of activity induced by these elements is especially effective in raising the level of excitation in the appropriate motor

Fig. 11.5. The composite nature of the releasing stimulus, analysed by means of models; the graphs show the proportion of male grayling butterflies responding by pursuit to the presentation of female dummies. a (solid curve), of different colour; b, of different shapes; c, of different sizes; d, moved in different ways. The dotted curve in (a) shows the effect of colour on the feeding response (redrawn from Tinbergen *et al.*, 1942).

centre. In view of the artificial nature of the experimental situation, and of the variability involved in behavioural experiments, one could not conclude from such experiments that any element of the normal stimulus situation is totally ineffective in contributing to motor excitation. It is sometimes possible, however, to carry out experiments in the context of a relatively unaltered and natural situation, and in this way to get more reliable evidence of the selective basis of the release. One of the most striking examples of this is the releasing stimulus for the act of copulation in the parasitic wasp *Mormoniella,* which is of classical simplicity. It consists of a lowering by the female of its horizontally extended antennae to a depressed position, out of reach of the male's mouthparts. Various other movements of the female are involved, but this antennal depression appears to be an indispensable condition. It can be simulated by antennectomy, and under these circumstances the male regularly attempts to copulate, but without success because the female fails to perform the movements normally associated with antennal depression, and essential for successful coupling. This example is of particular interest in that the releasing stimulus involves an absence of a stimulus, the female antenna, that was formerly there, indicating that certain stimuli may be inhibitory to the release of a motor pattern, in other words that the releasing mechanism is not based solely on excitation. Similar situations have been described for a number of other species, as for example in certain grasshoppers, where courting behaviour is inhibited by the songs of rival males, or in butterflies, where male markings, black with a white dot, inhibit the courting reactions of other males.

The fact that the ability of certain stimuli to evoke certain motor patterns is an inherited feature, that the innate releasing mechanism is really innate, has been convincingly demonstrated in a number of cases. Insects which have been raised in isolation are capable of responding to the complex succession of stimuli involved in courtship and mating; bees which have been raised in an incubator, after witnessing the tail-wagging dance of another member of the species indicative of the locality of a food source (see below), are able to fly, first time, in the indicated direction and for the indicated distance. This is not to say that no learning is involved in the discrimination of particular stimulus situations, but it does show that the basis of such discrimination is innately given as the counterpart of the fixed motor pattern, and, like that, presumably represents some particular configuration of neuronal architecture, but on the afferent rather than the efferent side.

(ii) The Steering of Motor Patterns. The performance of motor patterns is often orientated in relation to some feature of the total stimulus situation. Locomotion, for instance, elicited by the stimulus of a member of the same species as part of aggressive or reproductive patterns of behaviour, will be directed towards the releasing stimulus. Or locomotion, evoked endogenously, may be orientated in relation to a source of light, away from it or towards it or

at some specific angle to it; or in relation to gravity or to a gradient of humidity. The adoption of some particular posture may be similarly directed in relation to some particular source of stimulation, as when locusts bask in the rays of the early morning sun; or in relation to some particular object, as in the posture adopted by the praying mantis in relation to its prey immediately before the strike. Whatever the precise relation between the orientating stimulus and the motor pattern, and whether the orientating stimulus constitutes a part of the releasing situation, or is indifferent in relation to the evocation of the motor pattern, it is clear that there are basic mechanisms which enable the performance of motor patterns to be steered in relation to environmental stimuli, and the question arises as to the precise nature of these mechanisms.

It is in this context that the immense amount of work carried out during the early years of behaviour investigations, under the influence of the reflex approach to the study of behaviour, finds its rightful place. The results were usually based on a study of the reactions of insects to simple physical stimuli such as light, temperature, gravity, humidity, etc., but there is no reason to suppose that the visual orientation to a point source of light, for instance, differs in essence from orientation with reference to a member of the opposite sex, or an object of prey. What is important is not what is the object of orientation, but what is the mechanism by which orientation is achieved in relation to that object, and this has been established by such early work for a number of different types of reaction, in behavioural if not in neurophysiological terms.

The nature of the orientation mechanism appears to reflect in some measure the properties of the orientational stimulus. It has already been noted that with diffuse stimuli, for which a directional component may be lacking or weakly developed, kinetic mechanisms, characterized by an absence of orientation, may be of importance. When directional components, in the form of gradients of stimulation intensity, are sufficiently strong, orientation to such stimuli becomes possible, and it is usually achieved on the basis of a comparison of stimulation intensities, by a mechanism referred to as tropotactic. In many cases the comparison on which orientation is based involves bilaterally symmetrical receptors, so that an element of balance appears to be involved; if one receptor field is more strongly stimulated than the other, appropriate turning movements are initiated, and they continue until equality of stimulation is achieved, at which point the animal will be orientated with its long axis parallel to the gradient, and will move away from, or towards, the source of stimulation. The response of bees to attractive odours furnishes a useful example of tropotactic behaviour (Fig. 11.6(a)). Tested in a Y-tube olfactometer, normal bees will turn towards the source of attractive odour at the Y-junction; if, however, the antennae are immobilized in such a way that their tips are separated by less than 2 mm, they are unable to differentiate the simultaneous comparison between right and left, on which orientation is based, and they move

Fig. 11.6. Examples of the steering of motor patterns by environmental stimuli. (a) The reactions of bees to attractive odours. (i), normal bees tested in a Y-tube olfactometer select the air stream that carries the odour; (ii) bees, whose antennae have been fixed in such a way that the right antenna projects on to the left side, the left antenna on to the right side, select the odourless air stream; (iii) if the antennae are fixed in such a way that antennal tips are separated by less than 2 mm, the bees move indifferently into air streams with and without odour. (Data from Markl and Lindauer (1965.) (b) The "sun-compass" orientation of ants. At the point marked x, the ant was confined in a dark box for 2 hr. When freed, it assumed, in accord with the changed position of the sun, an incorrect direction on its way

(c)

(i)   (ii)

(d)

back to the nest, N (Markl and Lindauer, 1965 from von Frisch). (c) The graph shows the relation between the angle at which an ant was orientated to light, and the angle at which it subsequently orientated to gravity, when the light was switched off, and the platform swung from the horizontal to the vertical position; the angles were measured as the smallest angle between the track and the orientating stimulus, irrespective of direction. The correlation coefficient is 0.587, significant at the 0.001 level of probability. (Vowles, 1954.) (d) "Dances" of the honey-bee. (i) The pattern of the round dance. The dancer reports a source of food close to the hive. The dance followers maintain contact with the dancer by means of the antennae. (ii) The pattern of the tail-wagging run. The straight run between the two semi-circular ones is emphasized by wagging the abdomen. The straight tail-wagging run contains information about the location of the food source. (Lindauer, 1965, after von Frisch.)

indifferently into either side arm. If the antennae are fixed in such a way that the left antenna extends over to the right side, and the right to the left, the insects turn regularly into the odourless branch. It would seem that at the Y-junction the left antenna, in its artificial position, is less strongly stimulated than the right, and initiates turning movements which take the insect away from the source of stimulation.

Tropotactic mechanisms, involving an element of balance between symmetrically disposed receptor fields, have been demonstrated for humidity and temperature reactions, as well as for reactions to smell. Failure of orientation following unilateral extirpation of a sensory field, often coupled with so-called circus movements, which involve a continual turning towards, or away from, the intact side, will often provide evidence of the existence of tropotactic mechanisms. On this basis the orientation to light of certain insects appears also to involve a tropotaxis, but more usually reactions to stimuli with a strong directional component do not have to rely on comparisons of this type.

The neurophysiological basis of tropotactic orientation has not been elucidated, but it is one which can be envisaged in fairly simple terms. One could postulate that differences in the intensity of stimulation at bilaterally symmetrical receptors would be associated with corresponding differences in the level of input to bilateral centres of locomotion; this could, in turn, find expression in differences in the amplitude or frequency of limb movement, differences in "muscular tone" as postulated by earlier workers, to produce the appropriate turning component.

It is possible that orientation based on comparisons that are successive in time, rather than simultaneous, may be involved in the orientation of many insects to diffuse stimuli, although unequivocal experimental evidence is lacking. It is difficult to imagine that the known orientation of insects in flight to a source of smell could be achieved on the basis of a simultaneous comparison of stimulation intensities at bilaterally symmetrical receptors, in view of the likely effect of turbulence on the minute gradient which could be presumed to exist between right and left antennae. Under these circumstances a comparison of stimulation intensities at different points of the gradient, as it is traversed by the insect during flight, would appear to furnish a more promising basis for orientation.

Reaction to stimuli with a strongly developed directional component, such as light or gravity, are usually mediated by mechanisms referred to as telotactic, where the insect "fixates" the source of stimulation and moves away from it or towards it, or at some specific angle to it; in the latter case the reaction is referred to as a compass reaction or menotaxis. Compass reactions were discovered initially by observations of the behaviour of ants, which were found to maintain a fixed course during their foraging activities, by moving at a certain angle to the sun's rays. If the ant was confined in a black box at some point

along its route, and released after an interval of time, it would take up a course at an angle to its original course equal to the angle through which the sun had moved during the intervening period. Or if it was trapped at the end of its foraging run, it would set off for the return trip to the nest in a direction that showed a corresponding deviation from the correct one (see Fig. 11.6(b)).

Similar orientations in relation to gravity have been demonstrated in a number of insects. Ants, for example, can be trained to move to food from the centre of a vertical turntable, and to return to the centre, along any angle to the vertical, in the absence of light; only slight deviations from the target occur. Here the body is kept at a constant angle in relation to gravity rather than in relation to light.

Reactions of the type described obviously involve very much more than a simple reflex response to stimulation, explicable in simple neurophysiological terms. In the case of the light compass reaction, for instance, continual adjustments have to be made to the angle of orientation to compensate for movements of the sun, adjustments which fail to be made when the insect is imprisoned in the dark. Again, for both the visual and the gravity orientated responses, orientation can be switched through 180° for the return journey. The complexities involved are further emphasized by the remarkable ability of many insects to transpose a compass course from light to gravity and vice versa. The angle that is maintained in relation to a light source during locomotion on a horizontal surface is kept constant in relation to the gravitational force in the absence of light, when the surface is changed to a vertical one. For instance, the "escape run" of ants may be orientated in relation to light on a horizontal surface or to gravity on a vertical surface. If, during a run on a horizontal surface, the light is switched off and the surface swung to the vertical position, the run will continue at an angle to the gravitational field equal to that which previously obtained in relation to light (see Fig. 11.6(c)). A more natural example of such transposition is provided by the behaviour of foraging bees, following discovery of a source of nectar or pollen. On their return to the hive they communicate their discovery to other members by performing a special dance, the pattern of which may provide information about the direction in which the source is to be found, and about its distance from the hive. If it is within a hundred metres of the hive a "round-dance" is performed (Fig. 11.6(d)(i)), which signifies simply the presence of a source in close proximity. If the source is further away a "tail-wagging" dance is performed (Fig. 11.6(d)(ii)), in which the frequency with which the tail-wagging run is performed provides a measure of distance, while the orientation of the run on a vertical surface in relation to the gravitational field gives an indication of the direction of the source in relation to the sun.

The complexity of the mechanisms by which motor patterns are steered in these examples precludes a convincing neurophysiological interpretation in the

present state of knowledge, nor can there be much hope of bridging the gap between neurophysiology on the one hand, and behaviour on the other, until technical advances make possible an exploration of electrical activity in the neuropile in relation to functional organization. In the meantime, empirical studies of behaviour can do much to provide the firm foundation of quantitative information which must serve as the ultimate basis of a unitary interpretation. An outstanding example of the progress that can be made is furnished by the work of Mittelstaedt on the prey-catching strike of the mantis, as interpreted on the basis of information theory (Fig.11.7). When an insect moves within striking distance of the mantis it is visually fixated, the head of the mantis moving until

Fig. 11.7. Control pattern of prey localization in mantids. The prey is in a position which deviates from the body axis by the angle z, as monitored by the optic subsystem; the output from this system is an input variable, w, or "order", to the neck motor mechanism; this causes a movement of the head towards the prey, continuing until the deviation y, of the head from the body axis is equal to the deviation of the prey from the body axis; the deviation $x = z - y$, of the prey from the eye axis is then zero, and the insect is fixated. At this point, the "order" to the neck motor mechanism will be accurately proportional to the deviation of head, and hence of prey, from the body axis, and this "order" is used to determine the deviation, k, of the strike from the body axis. (Mittelstaedt, 1962.)

the image of the prey stimulates both eyes equally. At this point the strike is made by the forelegs, which are extended to the right distance and in the right direction to grasp the prey. The fixation is a relatively slow process, and appears to be controlled with little inertia or oscillation by a circuit of two closed loops each of two components; one between the visual mechanism and the motor mechanism of the neck; the other between hair-plate proprioceptors on the neck and the neck muscles. The strike, on the other hand, which is performed with lightning rapidity, and may be completed in less than 30 ms, is controlled by a single motor mechanism without feedback. There is, in other words, no mechanism for correcting errors in the strike once it has been released; its direction and extent is determined by steering mechanisms operative during the adoption of the strike posture, and these mechanisms in turn are based on input from visual and proprioceptive receptors. It is obvious that such detailed analysis of the components involved in the steering mechanism, and of their quantitative interrelationship, will serve as an indispensable basis for an eventual analysis in neurophysiological terms.

3. Learning

The extent to which the behaviour of insects is influenced by previous experience, the extent to which insects learn, has been much investigated. With insects, as with other animals, it is possible to distinguish a number of different kinds of learning, of which the simplest is the phenomenon of habituation to which reference has already been made. It is exemplified by the escape behaviour of the cockroach which, in the face of repetitive stimulation "learns" to ignore the stimulus; and in broader terms it finds expression in the general tendency for motor patterns to extinguish under conditions of repetitive stimulation. The neurophysiological basis of these effects have not been unequivocally established, and a number of different causes, including sensory adaptation and negative feedback from activated motor centres, may contribute to the total effect. This sort of learning is often of fairly brief duration, the memory short, though this is by no means invariably the case.

Other types of learning which are well exemplified by the behaviour of insects are "associative learning", which involves the building up of conditioned responses, and "trial-and-error learning". Many insects have been shown to be amenable to training of one kind or another, involving usually the association between stimuli which in themselves do not release any kind of response, with situations that do. Bees, for instance, can be trained to extend their probosces in response to odour, and are able to associate the colour of feeding places with the presence of food. Complex configurational stimuli can also be used as cues for food, and many insects have been shown to possess a high capacity for route learning in artificial mazes, a variety of stimulus modalities being put to use in identifying the correct route.

The olfactory conditioning which has been demonstrated in several species of insects bears a curious resemblance to the process of "imprinting", which is a feature of the learning process in many vertebrates. Here the full releasing stimulus is not innately given, but is in large part determined by experience during a particularly "sensitive" period of the life cycle. In the same way, the food or host choice of certain insects appears to be determined during early developmental periods by the substrate on which development occurs; transfer of choice from the normal host plant to an unusual one can be effected by raising the larval stages on the unusual type.

Evidence of a capacity for so-called "latent learning" is available from observations of the behaviour of insects in their natural environment, particularly in the context of route finding. The ability of the digger wasp to provision its nest with a succession of stung caterpillars, for example, is based on a capacity to learn the location of the nest, so that it can be found at the end of successive foraging trips. Such learning appears to be based on responses to a complex combination of features of the environment, and it seems that many insects build up a detailed spatial knowledge during preliminary orientation flights.

The one form of learning which has not been demonstrated in insects is so-called "insight learning", defined as the adaptive reorganization of the present content of experience in what may be described as "intelligent" behaviour. In view of the prevalence of other forms of learning in insects and of the fact that, with the exception of simple forms of habituation, the neural substratum of memory and learning in insects and in other animals remains obscure, it would perhaps be premature to dismiss the possibility of insight learning in insects. Even as they are, the demonstrated capacities for learning in the group are surprisingly extensive, and accord ill with the widely accepted concept of insects as the prime exemplars of a form of life based on innately determined behaviour. It would seem that despite the short life span which characterizes most members of the group, and despite the relatively small number of neural elements incorporated in their central nervous system, experience may play a prominent part in moulding behaviour patterns whose basis is innately given.

4. Conclusions

The question arises whether it is possible, against the background of this brief review of the main aspects of insect behaviour, to recognize features which could be interpreted as characteristic of insects as a group, and which could be seen as counterparts of other peculiarities of insect organization. The widespread occurrence of reactions to humidity could perhaps be seen as such. In terrestrial animals as small as most insects are, the threat to water balance posed by the terrestrial atmosphere is a serious one, and humidity will clearly constitute

one of the most important physical features of the environment. The subject will be discussed further in the last section of this book, and all that needs to be said here is that the possession of well-defined reactions to humidity, mediated by a variety of receptor types, effected by a variety of reaction mechanisms, undirected as well as directed, and regulated in a variety of ways, may be considered to represent a general characteristic of the group.

In considering other features of insect behaviour which could be classed as general characteristics, one may revert to the widely accepted view of the essentially instinctive nature of insect behaviour, and set against this the mounting evidence of a learning capacity which is little if at all inferior to that of other animal groups, not excluding vertebrates. If a substantial component of the behaviour of insects is in fact based on previous experience, then one may ask how the concept of insects as essentially instinctive has arisen. It seems possible that its source may lie in the apparent automatism of much of the motor activity of insects, which often has a robot-like appearance. This, however, may have little to do with whether or not the motor pattern is innately determined; what it may reflect rather is a characteristic which could stem from quite another feature of nervous organization, namely the lack of a strongly developed element of feedback. Insects are small animals, and their motor patterns are performed correspondingly quickly and at correspondingly high frequencies, as witnessed by the 30-ms prey-catching strike of the mantis, or the 0.5-ms wingstroke of the fly. Coupled with this, the nerve fibres of insects are unmyelinated, and therefore characterized by relatively low conduction velocities. It seems likely, therefore, that phasic feedback would in many cases be ineffective in producing corrections to a motor act, since the movement would have been completed before the appropriate information could be relayed through the central nervous system. As with the strike of the mantis, the movements would have to be largely predetermined, because by the time information is available concerning possible error, it will be too late to correct them. It may be this lack of fine adjustment of motor performance in relation to features of the environment, firmly imposed by properties of the nervous system, that produces an appearance of automatism; but such automatism does not imply a prevailing innateness, or an absence of learning; it is a simple defect of the motor act, imposed by the discrepancy between the speed with which the act is performed and the time required for effective feedback.

This concept receives some support from recent investigations of the control of flight in locusts, which have shown that the input from proprioceptors in the wings are not effective as phasic feedback, but that the information is integrated over a number of wing-beat cycles to produce a gradual adjustment in wing-beat frequency (Wilson, 1965). Another example of apparent lack of phasic feedback comes from observations on the grooming behaviour of certain insects. The wing-cleaning movements of flies normally consist of rubbing the hind leg over

the surface of the wing; if flies have their wings amputated at emergence, the normal leg movement occurs, indicating that the motor pattern is "fired off" and proceeds to completion in the absence of appropriate feedback. The suggestion is not intended that lack of feedback adjustment is a universal feature of insect motor patterns, and indeed, phasic feedback has been shown to play an important part in the performance of many motor patterns; but in some cases it is precluded by the rapidity with which the movement is made, and it would appear to play a relatively minor part in the motor patterns of insects as compared with higher animals. It seems possible that this may represent a general characteristic of insect behaviour, reflecting an aspect of the organization of its nervous system.

SECTION III

The Physiology of Reproduction and Development

INTRODUCTION

Two of the general characteristics of insect organization are of particular relevance to the physiology of reproduction and growth. One is the terrestrial mode of life, which has necessitated the development of internal fertilization and the provision of a strong and well water-proofed eggshell, and some of the special features of the reproductive system can be seen to provide a reflection of these requirements. The second is the possession of a rigid exoskeleton, which is of special significance in relation to the problem of growth; with such a skeleton, the animal is effectively contained within a cuticular prison of relatively fixed dimensions, and an increase in size can only take place by replacing one cuticular box with a larger one. The growth of insects tends, therefore, to be a discontinuous process, achieved in a series of steps marked by the shedding of the old cuticle in the process known as ecdysis. This stepwise transformation of small individuals into bigger individuals appears to have provided a suitable physiological basis for the far more spectacular transformations of metamorphosis, which characterizes many of the advanced orders of insects. Here the change is not from a smaller to a bigger individual, but from an individual of one kind to an individual of quite another kind, as in the transformation of a fly maggot into its winged adult, through the intermediary of the pupal stage. Here the individual organism is strikingly polymorphic, passing in the course of development through a succession of different forms, and the study of the mechanisms by which this metamorphosis is achieved has proved to be one of the most exciting in insect physiology.

REPRODUCTION

The main features of the reproductive organs of insects are illustrated in Fig. 12.1. The testes and ovaries are of mesodermal origin, while proximal parts of the reproductive system in both sexes derive from ectodermal invaginations, and

Fig. 12.1. The reproductive organs of insects. (a) Diagram of the female reproductive system (de Wilde, 1964 after Weber). (b) Diagram of the male reproductive system (de Wilde, 1964 after Wigglesworth). A.G., accessory gland; E.D., ejaculatory duct; M, mesodenia, O., ovarium; P, penis; S.P., spermatheca; T, testis; V.D., vas deferens; V.S., vesicula seminalis.

are provided with a cuticular lining. In most species the tracts of both sexes carry prominent accessory glands; in the female they secrete a variety of adhesive substances which serve as protective coverings for the egg, and in the male they produce, often in co-operation with glandular parts of the genital tract, the seminal fluid and the special sperm capsules, or spermatophores, in which the sperm is transferred to the female. In addition to the accessory glands, the female has one or more diverticula, known as spermathecae, in which sperm are stored after insemination.

a. Oögenesis

The ovary consists of a number (up to 2000) of egg tubes or ovarioles; the primordial germ cells are situated at the distal ends of the tubules, and as the oöcytes mature and grow, they move along them towards the oviduct. Three main types of ovary can be distinguished on the basis of oöcyte nutrition (see Fig. 12.2). In the panoistic type there are no special nutritive cells, other than the somatic follicular cells which surround each egg. In the polytrophic ovary, some of the undifferentiated oögonia develop to form nurse cells, or trophocytes, which contribute to the nutrition of the oöcyte during early stages of development, and accompany it on its passage down the ovariole. In the telotrophic type, the nurse cells do not accompany the oöcyte in its descent towards the oviduct, but remain at the apex of the ovariole; the nutritive materials which they supply are conveyed to the oöcyte through special nutritive cords.

During the process of oögenesis, materials required for subsequent embryonic development are incorporated in the substance of the egg cell as "yolk". The materials mainly involved are proteins, fats and carbohydrates, and they appear to be supplied by the trophocytes and follicular cells, taking the appearance of yolk spheres after they enter the oöcyte. Substantial quantities of nucleic acid must also be provided for the growth of the embryo, and in panoistic ovaries this appears to be supplied by extrusion of granules known as "chromidia" from the nucleus of the oöcyte itself. In the other types, the oöcyte may play little or no part in the synthesis of nucleic acids, which derive instead from the nurse cells.

The sequence of events involved in the vitellogenesis of a polytrophic ovary is illustrated in Fig. 12.2(b), which shows the early differentiation of trophocytes from oöcyte; the accumulation of chromidia coinciding with peak activity of the nurse cells; the regression of nurse cells during peak activity of the follicular cells; and the simultaneous appearance of fat droplets in the egg cytoplasm

The last stages in the formation of the mature egg involve the laying down of egg membranes. The thin vitelline membrane forms at the surface of the yolk, and on this is deposited the eggshell, or chorion, as a cuticular secretion of the follicular cells. The covering laid down by this single layer of cells rivals in complexity the cuticle itself, being composed in some insects of as many as seven distinct layers (Fig. 12.3(a)); the layers are distinguishable on the basis of composition, and proteins, lipids, polyphenols and mucopolysaccharides are principal constituents. It provides substantial mechanical protection, but its deposition at this stage raises a problem in relation to fertilization, because a massive shell of this sort would clearly provide an impassable barrier to the passage of spermatozoa; and it would also militate against an adequate supply of oxygen during the later stages of development when respiratory rates are relatively high. Both of these problems have been solved by the provision of a micropyle, a region of the egg surface at which the chorion is either sufficiently

(a)

(b)

thin to allow ready access of oxygen and of sperm, or where there are actual perforations of the eggshell.

The chorion of many species of insect has recently been shown to be intricately sculptured at the submicroscopic level as well as at the microscopic level, a phenomenon which appears to represent a respiratory adaptation of a rather paradoxical kind. One would normally think of dryness as a characteristic of the terrestrial environment, forgetting that such environments are subject to periodic wetting by rain or dew, and that what might be required for the eggs of many terrestrial insects, fastened to the substratum, and therefore liable to prolonged inundation, would be adaptations for aquatic as well as for terrestrial respiration. The sculpturing of the eggshell does in fact appear to represent a special device for ensuring adequate respiratory exchange during periods of immersion. It usually takes the form of one or more networks of chorionic processes, whose meshes entrap continuous and intercommunicating layers of air (Fig. 12.3(b)). The dimensions and physical properties of the system are such that it presents a strongly hydrofuge surface, and when submerged in water it will therefore constitute a physical gill, or plastron, providing a large air/water interface across which oxygen can diffuse to supply respiratory demands.

There remains to be considered the water-proofing of the insect egg; lipid components have been shown to be incorporated in the chorion, but they do not appear to constitute an effective barrier to the diffusion of water, for at the time when the development of the chorion has been completed, the eggshell is still quite permeable to water. It is not until later that there is a further secretion of lipid material, probably by the oöcyte itself, which passes into the space between the vitelline membrane and the inner layers of the chorion. At this stage the permeability decreases markedly, indicating that a layer of orientated lipid molecules has formed over the surface of the egg membranes. The permeability of this layer is affected by temperature in much the same way as is that of epicuticular wax layers, and this relation will be discussed further in Chapter 16.

The formation of the egg membranes completes the process of oögenesis, and the egg ruptures its follicle to pass into the oviduct for fertilization and eventual oviposition. The form in which eggs are deposited varies greatly from species to

Fig. 12.2. Oögenesis in insects. (a) Schematic illustration of the three main types of ovariole. A, panoistic type without nurse cells; B, polytrophic type with nurse cells interposed between successive oöcytes; C, telotrophic type with nurse cells confined to the apex (de Wilde, 1964). (b) Oögenesis in the polytrophic ovary of *Anopleura*. A, stage showing six undifferentiated oögonia; B, the six cells differentiated into five nurse cells and one oöcyte; C, the enlarged nucleus of the oöcyte giving off chromidia to the plasma; nurse cells beginning to function, next group of oögonia visible above; D, nuclei of follicular cells enlarge; nurse cells at height of activity; plasma with numerous chromidia; E, follicular epithelium active; nurse cells beginning to regress; chromidia formation ceases; fat droplets appear in plasma; F, follicular epithelium at height of activity; nucleus of oöcyte dissolves, chromosomes free in plasma at periphery of cell; yolk formation begins; G, ripe egg with reserve materials complete; follicle cells have laid down chorion; spindle of first maturation division formed (Wigglesworth, 1965 after Ries).

(a)

(b)

species; they are usually coated with secretions of the accessory glands, the details of whose activity have been investigated in a number of cases. In the cockroach quite different types of secretion are produced by the right and left members of the paired collaterial glands. The left gland, itself differentiated to form distinct regions, secretes a highly viscous liquid containing the protein which will form the structural basis of the oötheca, a β-glucoside of protecatechuic acid and a polyphenol oxidase. The secretions of the right gland contain a β-glucosidase, so that when the secretions mix in the oviduct, protecatechuic acid is liberated to serve as the substrate for the oxidase, and the quinones so produced form cross-linkages with the structural protein to give the hardened and darkened substance of the egg case (Brunet and Kent, 1955).

In mantids, the oötheca is formed from a viscous secretion of the accessory glands, which is beaten into a frothy mass that hardens on exposure to air and forms a vacuolated nest in which the contained eggs undergo embryonic development. It is uncertain to what extent the provision of these different kinds of egg pod is significant in relation to the protection of eggs from the desiccating influence of the terrestrial atmosphere. They are absent in many species which lay their eggs singly, and it is likely that the egg membranes themselves provide adequate water-proofing, and that the significance of the elaborate egg rafts formed in many species is to be sought in quite other terms.

b. Spermatogenesis

The testes comprise a number of tubular follicles, which contain the germ cells in different stages of development (Fig. 12.4(a)). At the apex of the follicle lie clusters of germ cells, or spermatogonia, interspersed with somatic nurse cells; as the spermatogonia move down the tubule they become invested in a layer of somatic cells, which form a cyst within which the germ cells undergo successive divisions to form the haploid spermatids. At the proximal end of the tubule, the spermatids undergo transformation to form spermatozoa, a process which involves the concentration of nuclear material to form the head, and the development of the characteristic flagellum. At this stage the spermatozoa break through the walls of their cyst and migrate to the seminal vesicles (see Fig. 12.1(b)), where they become densely packed to form a reservoir of mature sperm.

Fig. 12.3. Membranes of the insect egg. (a) The chorion of *Rhodnius*. Note the polygonal markings of the shell surface (pol mk), each with a follicular pit at its centre (f pt). The resistant exochorion layer is thickened at the base of each pit and at the surface ridges (r exo); s exo, soft exochorion layer; s end, soft endochorion layer; *Inset:* the detailed structure of the resistant endochorion layers; amb, amber layer; i pll, inner polyphenol layer; o pll, outer polyphenol layer; r end pr, resistant endochorion protein layer; l wx, primary wax layer (Beament, 1946). (b) Plastron of part of the egg shell of a fly, *Fannia armata* (Meig.). Air is held in a widely spaced meshwork as well as in a lower and finer meshwork, which shows high resistance to hydrostatic pressure. From unpublished scanning electron micrograph (x1550) courtesy of Professor H. E. Hinton.

(a)

(b)

(c)

c. Impregnation

The external genitalia of insects are enormousy complex, and the wealth of interspecific variation in structural detail has served as an invaluable basis for the taxonomy of the group. It seems likely that the difficulty of effecting a tight coupling between male and female genital tracts, the indispensable prerequisite for efficient internal fertilization in a terrestrial environment, must have posed a formidable problem for animals of the size of insects, and it is this that finds reflection in the intricate and highly specific configuration of their copulatory appendages. The "key-and-lock" principle suggested by their anatomical elaboration was originally thought to be of special significance in relation to the prevention of interspecific mating, but now it seems more likely that the main barrier to such mating occurs at the level of precopulatory courtship. The close and detailed fit of male and female genitalia should probably be interpreted rather in terms of the effective coupling which it ensures between the sexes during the period of sperm transfer, a process that may occupy several hours. In some species the sexes may actually become cemented together during copulation by special secretions of the male accessory glands.

The insertion of the penis into the female's reproductive tract appears to be effected by a hydraulic mechanism, fluid being pumped into the phallus by compression of the abdomen and, in some species, by the activity of a fluid pump situated at the posterior end of the ejaculatory duct (Fig. 12.4(b)); as erection proceeds, the penis gradually penetrates through the spermathecal duct to the spermatheca itself, into which the sperm is ejaculated.

In many insects the spermatozoa are introduced into the genital tract of the female as free suspensions in a seminal fluid. This fluid is elaborated in part by the accessory glands, and contains a high concentration of free amino acids, protein and carbohydrate. In others, the semen is enclosed in a membranous proteinaceous sheath produced, in the form of a compact spermatophore, from secretions of the accessory glands, often under the influence of other parts of the genital tract. This spermatophore is sometimes inserted by the male into the spermatheca of the female, but more usually it is deposited in the vagina, or in some cases simply dropped by the male during courtship, to be picked up by the female for deposition in the vagina. The precise form of spermatophores varies greatly from species to species (Fig. 12.4(c)), and their shape is often related

Fig. 12.4. Aspects of the male reproductive system. (a) Testis and follicle of a grasshopper. 1, general configuration of the testis; 2, single follicle with vas efferens; 3, enlarged view of follicle. A, apical cell; B, spermatogonia; C, spermatocytes; D, first maturation division; E, second maturation division; F, spermatids; G, spermatozoa (de Wilde, 1964 after Schröder). (b) Copulation in *Lygaeus equestris,* showing extension of penis of the male into the receptaculum seminis of the female (schematic); a, oviduct; b, receptaculum seminis; c, penis; d, vesicle containing fluid which is driven into the penis to extend and uncoil it (Wigglesworth, 1965 after Ludwig). (c) The spermatophores of different species of insect. A, *Blatella germanica*; B, *Sialis lutaria*; C, *Anabolia nervosa*; D, *Galleria mellonella*; E, *Pimpla instigator* (de Wilde, 1964 after Khalifa).

quite precisely to the shape of the female's vaginal chamber. They may be provided with apertures or tubes through which, after insemination, the spermatozoa can escape, to make their way to the spermatheca of the female. Mechanical pressure exerted by muscular contractions of the female's reproductive tract may contribute to the emptying of spermatophores, but in many insects it is only the neck of the spermatophore that is inserted into the genital tract of the female, the body remaining outside; here it seems probable that swelling of a gelatinous component of the spermatophore may serve to push out the contained seminal fluid. In some insects, the empty spermatophore case is eaten by the female, in others it appears to be digested by proteinases of the genital tract, and absorbed through its walls.

The mechanism by which spermatozoa, that have been deposited in the vagina, get to their eventual destination in the spermatheca appears to differ in different species. In some the sperm seem to move actively towards the spermatheca, possibly in response to a chemical stimulus; in others they appear to be transported passively by peristaltic contractions of the genital ducts. In *Rhodnius* it has been shown that such contractions can be induced by a component, possibly an *o*-dihydroxy-indolalkylamine, of the secretions of the male accessory glands.

d. Fertilization

Following impregnation, the spermatozoa are stored in the spermathecae until such time as they are required for the fertilization of eggs descending from the ovaries. The females of many insects mate only once, and the sperm from that first mating suffice to fertilize eggs during the whole of the egg-laying period, which may last for months and even years. The recruitment of sperm for fertilization appears, in some species, to be effected by the contraction of special muscles associated with the spermathecae, which pump out a batch of spermatozoa at the appropriate time. Once the spermatozoa have entered the oviduct near the micropyle of the egg, they may be guided to the micropyle by chemical stimuli; as soon as they have penetrated through the wax layer of the egg, a fertilization membrane is deposited between the wax layer and the oöcyte, and the spermatozoan loses its tail to transform into the male pronucleus. This combines with the female pronucleus to form the zygote, and thus the process of development is initiated.

DEVELOPMENT

The development of insects, as of other animals, from embryo to adult, involves two quite different processes, which can conveniently be discussed separately, though they take place concurrently; they are growth, which is referable to an increase in the number or size of cells, and differentiation, which involves a change in the pattern of their metabolic activity.

1. Growth

Growth is a readily definable phenomenon, which can often be measured in terms of cell number, or more simply in terms of size or weight. Since adult insects are small, the amount of growth that occurs during development from the egg is relatively slight, but since insects possess an external skeleton, it is much more markedly cyclical than it is in most other animals. The possession of a rigid cuticle effectively limits the size of an insect, and it can only grow in size by discarding its old exoskeleton and making a bigger one. Increase in size, therefore, tends to occur as a stepwise, discontinuous process; a phase of cell multiplication is followed by ecdysis, the moulting of the old cuticle, and an increase in size, with the deposition of a new cuticle conformable to the new size. So there is a succession of long intermoult periods, during which there is no change in linear dimension, alternating with short moults, during which a sharp increase in linear dimension occurs (see Fig. 13.1). If weight, rather than a linear measurement, is used as a measure of size, the discontinuities of the growth process are of a different kind; little increase in weight takes place during the short ecdysial phase, while the intermoult period is characterized by a substantial increase, reflecting the deposition of food reserves, and an increase in the size of cells (Fig. 13.1).

The number of moults which occur during development, the number of instars interposed between egg and adult, varies greatly from species to species. At one extreme is an insect like the mealworm beetle, which may have as few as four instars; at the other the firebrat, *Thermobia,* where the number of instars is indefinite as moulting continues in the adult, and as many as 60 may be passed

Fig. 13.1. The increase in size during growth; the hatched (lower) curve shows the increase in linear dimensions of the mealworm beetle; the upper curve shows the increase in weight of the stick insect, during successive instars. (Redrawn from Wigglesworth, 1965 after Teissier.)

through before death. There may be considerable variation in the number of instars within a given species, depending on genetic factors such as sex, or on external factors such as temperature or level of nutrition.

The succession, in the life cycle of insects, of a series of instars separated by ecdyses, imposes a cyclical pattern on the activity of epidermal cells. This involves the resorption of parts of the old cuticle, leading to a weakening of its structure, in preparation for the act of moulting; usually an increase in cell number, and an expansion of the epidermal surface following ecdysis; and the deposition by the epidermal cells of a new exoskeleton. These cyclical activities of the epidermis form the basis for the whole process of growth in insects, and consideration must be given to the details involved.

The structure of the cuticle has been briefly outlined in Chapter 1; it is illustrated again in Fig. 13.2, together with the cellular elements which contribute to its formation. These include:

(a) the epidermal cells;

(b) the oenocytes, characterized by massive accumulations of lipoprotein at the time of moulting;

(c) the dermal glands, also characterized by lipoprotein deposits, discharging to the surface of the cuticle through special ducts; and

(d) the tormogen and trichogen cells, which are responsible for the formation of sensory bristles, together with the neurones which innervate the sense organs.

All of these cell types have a common origin, arising by differentiation from cells of the epidermis; the last type of cell associated with the cuticle is mesodermal in origin; it is

(e) the haemocyte, which appears to be mainly responsible for the laying down of a basement membrane, that serves to support the epidermal elements. At the time of deposition of this membrane, when epidermal cells have completed their multiplication, the material contained in the haemocytes appears to be discharged to form a component of the substance of the basement membrane.

In the nymphal instars of *Rhodnius,* the cycle of epidermal activity is triggered by the act of feeding, and the sequence of events is illustrated in Fig. 13.2(b). Prior to the taking of a blood meal, the epidermis is in a relatively inactive state, but activation occurs soon after feeding. It involves a conspicuous enlargement of the nucleoli and the appearance of high concentrations of ribonucleoprotein in the cytoplasm, indicating that the cells are entering upon a phase of protein synthesis. On about the fifth day after feeding, the epithelial cells begin to divide, and a phase of intense mitotic activity follows, leading to an increase in the density of epidermal cells. At the same time, the conformation of the epithelial cells changes from squamous or cuboidal to columnar. At this stage the epidermal cells separate from the cuticle, and begin to lay down a new cuticle; they also secrete, into the space between the old and the new cuticle, a moulting fluid containing proteolytic enzymes and chitinase. Under the influence of these enzymes, the old cuticle begins to be digested away. The new cuticle is unaffected, because it is protected by the layer of cuticulin which is the first to be deposited, appearing as a delicate membrane that covers the folded surface of the epithelial cells, thus providing a capability for expansion once the old cuticle has been shed. The deposition of cuticulin is associated with a decrease in size of the greatly swollen oenocytes, and it is reasonable to suppose that the secretions of these cells furnish a raw material for the formation of the cuticulin layer.

Below the cuticulin is deposited the "homogeneous inner epicuticle" composed, as the cuticulin layer probably is, of a tanned lipoprotein complex. Below these there are in turn deposited the successive lamellae of the procuticle, at the same time that the definitive structure of the epicuticle is completed. Continuity between the epicuticle and the epidermis is maintained by the existence of pore canals, which in most cuticles traverse the lamellae of the protocuticle to terminate at the boundary between pro- and epi-cuticle. Through these pore canals, the epidermal cells secrete a material rich in polyphenols, that contributes to the formation of the epicuticle. They also constitute the route by which, just before moulting, waxes are secreted to ensure the water-proofing of the new cuticle, shortly to be exposed to the atmosphere.

The cuticular waxes are secreted as long, filamentous structures, 60—130 Å in

Fig. 13.2. Epidermal activity during the growth of insects. (a) Schematized diagram of the epidermis and associated structures. a, laminated endocuticle; b, exocuticle; c, epicuticle; d, bristle; e, pore canals; f, duct of dermal gland; g, basement membrane; h, epidermal cell; i, trichogen cell; k, tormogen cell; l, oenocyte; m, haemocyte adherent to basement membrane; n, dermal gland. The sense cell and axon of the bristle have been omitted. (Wigglesworth, 1965.) (b) Schematized diagram showing changes in the density of epidermal cells during the moulting cycle, associated with the resorption of old and the deposition of new cuticle in fourth and fifth instars of *Rhodnius*. 1, cuticle of fourth instar; 2, moulting fluid; 3, cuticle of fifth instar; 4, epidermal cells; 5, basement membrane (redrawn from Wigglesworth, 1959 and Locke, 1964).

Fig. 13.2. (cont.) Lipids of the insect cuticle. (c) Diagram of the structure of two phases of lipid/water liquid crystals; above, transverse and below, longitudinal sections; (i) the middle phase; (ii) the complex hexagonal phase (Locke, 1964 after Luzzati and Husson). (d) The structure of the surface of an insect. Not all insects have all the illustrated components; the cement is frequently lacking, and wax blooms would then form directly over the wax canals, or the waxes may be liquid and mobile, in which case blooms would not form (Locke, 1964).

diameter, and visible in electron micrographs. These filaments appear to represent lipid/water liquid crystals, which may exist in a number of different phases (see Fig. 13.2(c)). The "middle phase" crystals have the hydrophobe carbon chains directed towards the interior of the filament, with the hydrophil, polar groups towards the lipid/water interface at the surface of the filament. In the "complex hexagonal phase", the filaments are double-walled, with hydrophil polar groups both inside and outside the filament. These liquid crystals appear to be discharged from the tips of the pore canals, and to penetrate the epicuticle through minute wax canals that extend through the cuticulin layer to the surface. It is envisaged that the middle phase crystals are continuous with a surface monolayer of lipid (see Fig. 13.2(d)), orientated with polar groups towards the hydrophil polyphenol substratum of the cuticulin layer. Lipids which cannot be accommodated in the monolayer are extruded to its outer surface, where they form a less well-orientated layer, mixing with the cement which is being secreted from the dermal glands in a complex which may serve to protect the monolayer. In many species of insect wax-blooms, composed of thin sheets, or plates, of orientated lipid molecules may project from the general surface, as shown in the diagram.

This recent work has provided a satisfactory explanation of a phenomenon which has long puzzled insect physiologists, namely the secretion of insoluble lipid material through a water-impregnated cuticle. It should be mentioned,

however, that it does so on the basis of the participation of lipid molecules which have one end polar and hydrophil, the other non-polar and hydrofuge. Only a small proportion of the cuticular lipids of insects are, in fact, of this type. It would presumably be possible to accommodate non-polar lipids within the hydrophobe core of a middle-phase crystal, but evidence for such an arrangement is lacking.

The mechanism by which the lamellae of the procuticle are deposited below the epicuticle has not yet been unequivocally established, but it appears to involve the secretion of cuticular material (chitin and protein) from the base of the finger-like processes that project into the pore canals from the epidermal cells. The lamellar structure seems to be associated with a rhythmic activity of the epidermal cells, most of the material being deposited during the night. Only that part of the procuticle which is destined to become tanned exocuticle is laid down at the time of moulting, the soft endocuticle continuing to form during the intermoult period, right up to the time of the next moult.

During the time when the epicuticle and presumptive exocuticle are laid down, the bulk of the old cuticle is being digested away by enzymes of the moulting fluid, and the fluid plus dissolved digestion products are resorbed from the space between the old and the new cuticle. Precisely how this is achieved has not yet been determined, but it is possible that absorption takes place through the wax and pore canals.

As the insect emerges from its old, attenuated cuticle, which splits along ecdysial lines of weakness, it swallows air to expand its bulk. The swallowing of air, together with tonic contractions of the muscles of the body wall, ensures the development of substantial haemolymph pressure, and under the influence of this pressure, the soft and extensible new cuticle stretches to take up the conformation of the succeeding instar, thereby reducing the density of epidermal cells as illustrated in Fig. 13.2(b). Once this has occurred, the cuticle begins to darken and harden by the mechanism outlined in Chapter 1. The polyphenol tanning agents appear to be discharged from the tips of the pore canals, and to diffuse inwards, so that darkening and hardening occurs first in the cuticle which adjoins the epicuticle, proceeding inwards from there.

Growth of the epidermis is thus seen to be a particularly complex phenomenon, because it is restricted by the presence of a rigid exoskeleton. It involves the partial dissolution of that skeleton, and the deposition of a new one, once expansion of the epidermal surface, based on an increase in the number of epidermal cells, has occurred. In tissues other than the epidermis and its derivatives, this complication does not arise, and here all that need be involved is a multiplication or growth of cells, and their differentiation along lines appropriate to the particular tissue concerned. It should be emphasized, however, that the growth of tissues is not based on a simple process of cell division. In many insects, there may be little or no cell multiplication during

larval instars, and increases in the surface area of the epidermis are associated with an increase in the size, rather than the number, of epidermal cells. Where multiplication of cells does occur, the rate of cell division during periods of growth is generally excessive, so that many more cells are formed than are required. The surplus of cells undergoes histolysis, the nuclei disintegrate to form "chromatin droplets", and the products of histolysis are either assimilated by surviving sister cells, or they diffuse into the haemolymph to become part of the general metabolic pool, while cellular debris may be cleaned up by phagocytes of the haemolymph (see Chapter 3). At any moment, therefore, the number of cells present reflects the state of balance between mitotic activity on the one hand, and cell destruction on the other; during later stages of the growth cycle, histolytic processes may outstrip mitotic recruitment, so that the number of cells actually decreases.

2. Differentiation

In insects, as in most other animals, growth in size is usually associated with a change in form. One instar does not differ from the next simply in that it is bigger; at the least there will be some change in the relative proportion of parts, and often profound differences in the details of structural organization are involved, a differentiation of one instar in relation to its predecessor. The degree to which such differentiation occurs at moulting to the adult forms the basis of a subdivision of the class into three main groups. In the Ametabola the change from young to adult is a gradual one, achieved in the course of a succession of nymphal instars. The point at which these insects become sexually mature does not necessarily mark the cessation of growth and moulting, nor is it associated with a change in form which could rightly be described as metamorphic. Insects in which active nymphal stages are transformed directly into the adult form without the intervention of a quiescent pupal stage are classed as Hemimetabola; here the change in form at the last moult to the sexually mature adult is usually substantial in comparison with the change between successive nymphal instars. Species where a comparatively inactive non-feeding pupal stage is interposed between the immature forms, normally referred to as larvae, and the sexually mature adult are included in the Holometabola, showing complete metamorphosis. What is ultimately distinguished in this classification is the magnitude of the metamorphic change that occurs when the insect moults to the sexually mature form; the change is small in the Ametabola, great in the Holometabola and intermediate in the Hemimetabola. The difference is clearly one of degree, and it has been found to reflect a corresponding difference at the level of developmental physiology, to which attention must in the first place be directed.

The earliest stages in the development of insects are characteristically regulative, in the sense that products of the first few cell divisions are totipotent,

each capable of giving rise to a fully-developed organism if other division products are destroyed or eliminated by microsurgical techniques. As development proceeds, the embryo becomes progressively more of a mosaic, so that extirpation of cells in one part of the embryo will result in the development of individuals with deficiencies in the corresponding region. The different cells, exposed to different influences as a result of the developmental process, become determined to differentiate along certain lines, and are therefore no longer capable of substituting for cells determined to a different fate. The process of determination involves the suppression, often irreversible, of certain potentialities, and the manifestation of others, out of the total represented in the genotype. Initially, for example, all cells would have the prospective capability for the production both of muscle proteins and of cuticular proteins; but in the cells which are, in the course of development, induced to form muscle cells, the capacity to produce cuticular proteins is suppressed or lost, while in prospective epidermal cells, the capacity to produce muscle proteins is suppressed or lost. Each type of cell, by emphasis on this or that metabolic pathway, develops its own particular pattern of metabolism, ensuring the production of this or that particular reaction product, a structural protein, a hormone or a digestive enzyme, appropriate to the performance of its function in the organism as a whole. In the course of embryogenesis, there will be formed a corresponding diversity of tissues, serving as the basis of corresponding organ systems.

The post-embryonic development of the Ametabola can be seen as a simple extension of such a process of progressive differentiation; it involves a gradual increase in the size of the insect, with perhaps some slight changes in the relative proportion of its parts. This would be capable of being achieved without a change in the nature of the activity of particular types of cells; the epidermal cells would continue to secrete the same type of cuticle, the muscle cells the same type of muscle protein at all stages of development. Only the sex cells come under what could be described as a metamorphic influence; during early stages their differentiation is completely suppressed, but as the insect nears the completion of development, the suppression is lifted, and the cells proceed to differentiate to their functional stage in the sexually mature insect.

In the Hemimetabola the situation as far as the sex cells are concerned is much the same, but here the last moult to the sexually mature adult is associated with a substantial change in the general form of the insect. *Rhodnius* is a member of this group whose development has been particularly carefully investigated. In this insect the metamorphic moult is characterized, among other things, by the formation of large membranous wings in place of the inconspicuous wing lobes of earlier instars, and the development of external genitalia. These formations express the morphogenetic movements of corresponding regions of the epidermis, but even in parts which are unaffected by such changes in surface conformation there is a marked difference in the type

of cuticle laid down before and after the metamorphic moult. The nymphal cuticle is thick, soft and extensible, and the surface is beset with small plaques that bear the sensory bristles (see Fig. 13.3(a)). The adult cuticle, on the other hand, has fewer bristles of a different type, and is thin and heavily sclerotized; distension of the abdomen during feeding is provided for not by extensibility of the general cuticle, but by the unfolding of lateral pleats of soft cuticle. The pattern of pigmentation also differs considerably, with nymphal pigment spots confined to the postero-lateral margins of the abdominal sclerites, while the adults have larger spots at the antero-lateral margins. This furnishes a good example of the way in which profound changes in the general appearance of an insect can be based largely on changes in the activity of epidermal cells. From the point of view of developmental biology the phenomenon may be said to represent a retention of pleuripotency on the part of the epidermal cells. The cells have not been irrevocably determined to the laying down of a particular type of cuticle, but have retained a capacity to lay down two different types, so that the same cell that at one moult produces cuticle of one sort, at the next lays down quite a different sort; a potentiality which was suppressed at the first moult finds expression in the next.

That the capacity to lay down nymphal and adult cuticle is present latently in the same cell is well illustrated by experiments involving injury to the epidermis. If a section of cuticle is injured by burning, for instance, the uninjured cells at the margin of the wound divide and spread inwards to repair the damage, and as they do so they carry with them their special characteristics. If the burn passes near a black pigment spot, the proliferation and inward spread of the corresponding cells is reflected, at the next moult, in a corresponding extension of the pigment spot, as shown in Fig. 13.3(b); or if one pigment spot is burnt out, its place at the next nymphal moult will be taken by cells which have spread from adjacent non-pigmented areas. But if the following moult is to the adult, then the situation changes, for the pigment spots of the adult occupy places which in the nymphs are unpigmented, and vice versa. Hence, where the nymph had an extended pigment spot, the adult will have unpigmented cuticle in place of its normal pigmentation, and where the nymph lacked a pigment spot, there the adult will have an extra pigmented area.

Such experiments show that the capacity to produce the adult pattern is present in the nymphal cells, and if they are induced to divide at the nymphal stage, they distribute their potentiality to their daughter cells; but the adult pattern remains latent, to find expression when metamorphosis occurs. If this is so, one would expect that, provided "metamorphic" conditions could be provided, nymphal cells might exhibit adult characteristics at any stage in nymphal development, and conversely, that adult cells might be induced to exhibit characteristics of the nymphal cells, provided they could be subjected to a nymphal environment. Within limits this can, in fact, be shown to be the case.

Fig. 13.3. Aspects of the differentiation of cuticle. (a) Nymphal and adult cuticle of *Rhodnius*. A, transverse section of abdomen of unfed fifth-stage nymph; A′, the same immediately after feeding. B, transverse section of abdomen of unfed adult; B′, the same immediately after feeding. C, detail of lateral pleat in abdomen of unfed adult; D, detail of extensible lower wall of the pleat; E, detail of "hinge-line" in tergites. F, longitudinal section of abdominal tergite of fifth-stage nymph; G, longitudinal section of abdominal tergite of adult (Wigglesworth, 1959). (b) The effect of injury on the development of pigment spots in nymphal and adult *Rhodnius*. A, third, fourth and fifth tergites of a normal third-stage nymph; the broken lines at "a" and "b" show the regions burned. B, corresponding segments in the fifth-stage nymph resulting. C, corresponding segments in the adult resulting (Wigglesworth, 1959).

If, for example, the endocrine organs responsible for the maintenance of the nymphal state (see next chapter) are implanted below the cuticle of a fourth instar nymph, then when the insect moults to the adult form, the cuticle covering the implant will be of the nymphal type, surrounded on all sides by normal adult cuticle.

While the metamorphic changes associated with the activity of the epidermis are the most spectacular, and have been the most carefully studied, they are by no means the only ones involved in the metamorphic moult. The formation or elaboration of movable appendages, like wings and external genitalia, will clearly be associated with corresponding changes in musculature, which may involve both degenerative and regenerative processes. Other internal organs, like the tracheal system, the nervous system or the excretory system tend, by contrast, to be relatively little affected by the metamorphic moult.

While the metamorphosis of the Hemimetabola may be sufficiently spectacular, as in the transformation of a virtually wingless young to the fully-fledged adult, the changes involved are trivial compared with the total transformation that occurs during the metamorphosis of holometabolous species. In them the immature insect may be a soft, white and virtually featureless maggot, anatomically and physiologically adapted to a simple mode of life; this larva will develop, through the pupal stage, to its winged and six-legged, firmly sclerotized and finely sculptured adult counterpart, quite another animal with a different and much more complex mode of life. The change from larva to adult could in this case be thought of as being too far-reaching to be accomplished in a single moult, hence the interposition of a quiescent pupal stage, devoted to the cellular reorganization involved; and too drastic to find a basis in the capacity of epidermal cells to differentiate first along immature and later along adult lines. Instead, the task of adult reconstruction has in this group been delegated to special embryonic cells, whose development is totally suppressed during larval life. Clusters of such cells form the imaginal buds, or histoblasts, which can be found dispersed among the larval tissues, most conspicuously in regions where adult appendages are destined to make their appearance as antennae, mouthparts, legs, wings and external genitalia. There is thus no question of a change in the direction of development of epidermal cells from larval to adult patterns, but rather of an almost complete replacement of larval epidermal cells by cells produced from imaginal buds, and differentiating along adult lines. In these insects, too, the striking difference between larval and adult modes of life render many of the internal organs of the larva unsuitable to perform their function in the context of the adult organization, and a need arises for the partial or total replacement of musculature, alimentary canal, salivary glands, fat body etc. The pupal stage is therefore a period of intense histolytic activity, which furnishes the raw materials for the histogenesis of imaginal buds.

Three main types of developmental process seem thus to be involved in the

mechanism by which the adult stage is attained in insects. One is a progressive differentiation of somatic cells and tissues towards the adult condition. Another involves the retention of embryonic characteristics, mainly by cells of the epidermis, which enables a switch to be made in the pattern of development at the time of metamorphosis, so that cells which had previously disposed themselves to form the nymphal surface, and secreted nymphal cuticle, now become the source of morphogenetic movements that tend to the formation of the differently disposed adult surface, covered by the adult type of cuticle. In the third type, two sets of cells are formed during early stages of development, one of which is responsible for the formation of the larval organization, while the other is held in reserve, its differentiation suppressed during larval life; activity is resumed in these cells some time before the metamorphic moult, and on the basis of their growth and differentiation the adult organism is formed to replace the crumbling fabric of larval tissues. As has already been indicated, these three processes are involved to different extents in the development of the three groups of insects, the first serving as the main basis of ametabolan, the second of hemimetabolan and the third of holometabolan development. It must be emphasized, however, that the distinction that can be drawn in these terms is again one of degree; histoblasts, for instance, are a feature of the development in many Hemimetabola as well as of the Holometabola, while epidermal pleuripotency is involved in the metamorphosis of a number of Holometabola as well as in that of the Hemimetabola. A capacity to manifest all three types of developmental process appears to be shared by all members of the class, but the emphasis is shifted from one to another in accord with the demands made in terms of reconstruction at the metamorphic moult. The feature that is common to all is a suppression of adult characteristics during early stages of development, and the suppression of larval characteristics at metamorphosis, and the nature of the control systems responsible for this change will be discussed in the next chapter.

3. Diapause

One aspect of the growth and development of insects that must be briefly mentioned is the phenomenon of diapause, or more generally, of arrested development, which is an important feature of the life history of many species of insects. It appears to be essentially a mechanism for limiting the occurrence of delicate morphogenetic processes to periods during which environmental conditions are favourable; or, alternatively, for synchronizing the life cycle with seasonal fluctuations of climate in such a way as to ensure that an abundance of food will be available for active stages of the life history.

In many species, arrest of development, or quiescence, may occur as a direct result of inclement conditions; poor food, for instance; or drought and

desiccation; or excess moisture; or low temperatures. In others, however, a prolonged arrest of development may occur, at one or another stage of development, quite irrespective of the current environmental situation. The timing of diapause in such species is still linked to seasonal changes, but more remotely, and any one of a number of different environmental factors may be involved in the induction of diapause. Of these, photoperiod appears to be one of the most important, and Fig. 13.4(a) illustrates the relation between day-length and the intensity of diapause in different species, as measured by the proportion of individuals that exhibit developmental arrest. In one of the species, larval diapause is induced over a narrow range of day-lengths between 10 and 14 hr; in another, exposure of eggs to day-lengths in excess of 16 hr causes the production of a high proportion of diapause eggs; and in another the proportion of diapause pupae increases gradually as the period of daylight increases from 0 to 12 hr, and then falls again to zero.

Diapause in temperate species of insect can usually be seen as a mechanism for surviving during the winter season, when temperatures drop to levels that are too low for normal developmental processes. It is not surprising, therefore, that exposure to low temperatures is one of the best ways of breaking the diapause. The mechanism of this effect seems to be based on the fact that the corresponding physiological process, which may be different in different insects but which leads to what may be called the breaking of diapause, occurs at high rates only when the temperature is low. This is illustrated in Fig. 13.4(b) for the embryonic development of an Australian grasshopper. Curves on the left show the proportion of eggs in which the breaking of diapause is completed in 60 days, as determined on samples originating in different parts of the country. It is highest at temperatures between $10°$ and $15°$, falling to lower values at lower temperatures, and to zero at about $25°$. At temperatures which ensure rapid breaking of diapause, however, the rate of normal morphogenesis is negligible, as shown by curve B. To effect rapid development, eggs must therefore be exposed first to low temperatures, which will hasten diapause development, and then to high temperatures, which will allow morphogenesis to proceed rapidly. The phenomenon of diapause seems, in other words, to be based on an inhibition of some part of the developmental process, an inhibition which can only be released by a reaction that has a temperature coefficient different from, and usually lower than, that of morphogenesis. Where the two temperature curves show a degree of overlap, as in the illustration, development will proceed slowly to completion at a constant intermediate temperature; where there is no overlap, as is the case with eggs of the silkworm, development cannot occur at intermediate temperatures; and where the optima are so close together that overlap is extensive the existence of diapause may be difficult to establish experimentally.

The environmental factors which induce and break diapause have been

(a)

(b)

identified for a number of different species of insect, but the mechanism by which they exert their effect is still in doubt. It seems to be generally agreed that the immediate cause of diapause is lack of growth hormone, a substance whose source and properties will be described in the next chapter; but precisely how the arrest of neuroendocrine secretion is ensured has not yet been established.

Fig. 13.4. The relation between diapause and certain environmental factors. (a) The effect of photoperiod on the incidence of diapause in some species of Lepidoptera. Ordinate: percentage of individuals entering diapause; abscissa: hours of light in 24 hr. A, *Acronycta rumicis* (after Danilyevsky); B, *Grapholitha molesta* (after Dickson); C, *Pyrausta nubilalis* (after Beck); D, *Bombyx mori,* bivoltine race (after Kogure) (Wigglesworth, 1965). (b) The influence of temperature on the breaking of diapause and on morphogenesis in the embryo of *Austroictes cruciata*. A, the ordinate shows the proportion of eggs to complete the breaking of diapause during 60 days at the specified temperatures; complete line: eggs from South Australia; broken line: eggs from Western Australia. B, the ordinate shows the proportion of post-diapause development completed each day at the stated temperatures (Andrewartha and Birch, 1954).

NEUROENDOCRINE CONTROL SYSTEMS

Brief mention has been made in earlier chapters of the control by endocrine secretions of somatic processes like digestion and excretion, and of neuro-muscular processes like activity rhythms. It seems likely that as more of the details of such physiological processes become known, so will the study of their neuroendocrine control systems become of increasing importance. It is in the fields of developmental and reproductive physiology, however, that neuro-endocrine regulators play their most spectacular role, and it seems appropriate, therefore, to have delayed a detailed discussion of the systems involved till now, when the main features of reproductive physiology have been outlined. The control of growth and metamorphosis by the neuroendocrine system of insects has, indeed, provided one of the most intriguing problems for insect physiologists since the discovery by Kopeč, in the early decades of this century, that the moulting of insects is under the control of a circulating hormone. A great deal of outstanding experimental work has been done to elucidate the factors involved, and on the basis of results obtained it has been possible to formulate a satisfactory interpretation of the general situation, though much remains to be done at the level of specific detail.

1. Neuroendocrine Control of Growth and Development

In order to provide a broad framework for the discussion that follows, it will be useful to review briefly the main features of the neuroendocrine system concerned, and to outline its mode of action in general terms. The components of the system are illustrated diagrammatically in Fig. 14.1, and they are seen to comprise four main parts:

(a) clusters of neurosecretory cells in the brain, whose secretions are passed along nerve axons to

(b) the corpora cardiaca, constituting a neurohaemal organ associated with the dorsal blood vessel; through this organ the brain secretions are passed into the bloodstream to exert an activating influence on

(c) the thoracic glands; these glands then secrete a hormone called the

Fig. 14.1. A general outline of components of the neuroendocrine system concerned with the control of growth. c.a., corpus allatum; c.c., corpora cardiaca; d.a., dorsal aorta; n.c., neurosecretory cells of the protocerebrum; n.c.c., nervi corpora cardiaca; t.g., thoracic glands.

moulting hormone, or ecdysone, which initiates the cycle of growth that has been described in the previous chapter;

(d) the last major component of the complex is the corpus allatum, closely associated with the corpora cardiaca. The activity of this gland is under the control of the brain, and it produces a hormone known as the juvenile hormone, which is passed into the bloodstream; when the hormone is present in sufficiently high concentration at the time of moulting, juvenile, or larval, characters are manifested, while in its absence, the adult characteristics make their appearance.

Against this background, the general growth and development of insects may be seen as governed by the cyclical activity of the neurosecretory cells of the brain. The hormone produced activates the thoracic gland, which secretes moulting hormone to induce a corresponding succession of moults. During early stages, the activity of the corpus allatum ensures the appearance of juvenile characters; with decreasing activity and a corresponding decline in the titre of

juvenile hormone, pupal, and eventually adult, characters supervene. On the basis of this interpretation, interest will centre on the nature of substances released by the various glands, the nature of their action, and the mechanisms by which the secretory activity is triggered to produce co-ordinated cyclical activity in the system as a whole; these aspects will be discussed in the sections that follow.

a. The Neurosecretory Cells and the Corpora Cardiaca

The neurosecretory cells are situated in the median dorsal region of the brain; at certain points in the cycle of their activity, the cell bodies contain an accumulation of secretory material in the form of minute granules with characteristic staining properties, 1000–3000 Å in diameter, as shown in Fig. 14.2(a). This material can also be detected in the axons which pass back towards the corpora cardiaca, and if the axons are ligatured or severed, neurosecretory material accumulates above the point of interference (see Fig. 14.2(b)), suggesting that the granules are transported from cell body to corpus cardiacum by bulk flow along the axons. At the same time that neurosecretory material dams up at the level of ligatured axons, the store of such material which is normally present in the corpora cardiaca disappears, and it seems that this organ is mainly concerned with the storage and release of brain hormone.

The role of brain hormone in moulting was demonstrated by the early experiments of Kopeč, who showed that when larvae of the gypsy moth were deprived of their brains 10 days or more after the last larval moult, pupation

(a)   (b)

Fig. 14.2. Neurosecretory cells of the protocerebrum. (a) A neurosecretory cell from the locust. a, cell body; b, nucleus; c, neurosecretory granules; d, axon (redrawn from Highnam, 1961). (b) Schematized drawing of fresh preparation of the fused nervi corpora cardiaca and nervus recurrens of the blowfly three days after ligature. a, proximal part of nerve; b, axon with swellings of neurosecretory material; c, bulk accumulation of neurosecretory material; d, ligature; e, distal part of nerve, lacking neurosecretory material (drawn from photomicrograph of Thomsen, 1954).

occurred, and brainless but otherwise normal pupae and adults were formed. If the extirpation was carried out less than 10 days after the moult, however, the caterpillars failed to pupate, although they continued alive for a long time. Larvae which had been tightly ligatured behind the thorax pupated if the ligature was tied after the 10th day, but before that only the front half underwent pupation. These experiments showed clearly that a blood-borne factor was involved in the induction of the moult, and that there appeared to be a critical period at about the 10th day, during which the secretion was liberated.

Later experiments have amply confirmed these original findings, and have served to implicate the neurosecretory cells of the protocerebrum specifically in this action. If this region of the brain, for instance, is implanted into insects which have been decapitated before the critical period, the implants will activate the system, and moulting will take place in insects which would otherwise not have moulted.

The chemical nature of the neurosecretory hormone has not yet been unequivocally established, but it seems likely that it is a peptide, since it can be inactivated by bacterial proteinase. The stimuli which cause its liberation appear to vary greatly between different species of insect; in some they are closely linked with the act of feeding, which is not surprising in view of the heavy demands on raw materials which are made by processes of growth; in these, the effect seems to be mediated, at least in part, by stretch receptors associated with the alimentary canal, monitoring the distension caused by the presence of food. But in other species other factors appear to be involved, and the situation does not lend itself to convincing generalization.

b. The Thoracic Glands

In the course of time it became apparent that it was not just the liberation of brain hormone from the corpora cardiaca that caused moulting. If certain species of insect were ligatured, for instance, in such a way that the head was isolated from the thorax before the critical period, neither the anterior nor the posterior portions moulted. It was subsequently discovered that a two-stage process is in fact involved, with the brain hormone exerting its effect on the thoracic gland, and with this in turn producing and liberating a moulting hormone.

The thoracic glands originate as ectodermal invaginations of the head region, but they take up different positions and assume different forms in different insects. In some they remain in the head as compact "ventral glands"; in some they become associated with the corpora cardiaca/corpus allatum complex as a "Weisman's ring"; in some they form a loose network of cells in the thorax, while in many they become closely associated with the tracheal system. Whatever their position, when cells of the thoracic gland come under the influence of the brain hormone, they enter upon a cycle of activity involving an increase in the size of the nuclei, which often become extensively lobulated, and

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an increase in the amount of basophilic material in the cytoplasm (see Fig. 14.3(a)). The precise significance of these changes has not yet been established, but it appears that the cells become involved in the synthesis of the moulting hormone itself, together with a variety of mucoproteins, glycoproteins and other substances with which the hormone appears to be associated. The hormone itself has been isolated in crystalline form, with a yield of about 5 mg from 100 kg of

0·1 mm

(a)

(b)

Fig. 14.3. The thoracic gland. (a) Changes in the histology of the thoracic gland during its cycle of activity in *Rhodnius*. A, the inactive stage in unfed fifth-stage nymphs; B, the active stage in fifth-stage nymphs 10 days after feeding; C, the phase of regression in adults one day after moulting, showing numerous haemocytes around the disintegrating nuclei (Wigglesworth, 1952). (b) The structure of the moulting hormone α-ecdysone (from Karlson and Sekeris, 1966). (c) The relationship between the quantity of moulting hormone injected into last instar larvae of *Chironomus tentans* and the degree of "puffing" of locus I-18-C on the salivary gland chromosome 3 hr after injection. The sketches show the appearance of the four classes of "puffing" distinguished, whose frequency distribution among larvae injected with the stated quantities of hormone are illustrated in the histograms below. The shift towards higher levels of "puffing" with increasing dose is clearly shown, n = number of larvae used (Clever, 1963).

(c)

silkworm pupae. It has recently been identified as 2,3,14,22,25-pentahydroxy-Δ7-cholestene-6-one, and its structure is illustrated in Fig. 14.3(b). 7.5 mμg of the purified material is capable of inducing puparium formation in the isolated abdomen of blowfly larvae. Following production of the moulting hormone and associated materials, the cells of the thoracic gland enter upon a phase of regression, and revert to their resting condition.

The primary effect of the release of moulting hormone from the thoracic gland is an "activation" of all cells which are involved in the processes of growth and moulting, notably the cells of the epidermis. There is an enlargement of the nucleoli, a mobilization of ribonucleoprotein and an increase in the rate of protein synthesis. There is also an increase in the number of mitochondria, which must in itself involve a substantial synthesis of their protein and lipid components. The mechanism by which this activation of the synthetic machinery is achieved is still uncertain, but recent observations suggest that a direct action on the genetic material may be involved. It has been shown that within 10 min of the injection of moulting hormone, in quantities corresponding to about 10 molecules per haploid set of chromosomes, it is possible to distinguish the appearance of "puffs" on salivary gland chromosomes, an effect which may reflect the activation of particular genes (see Fig. 14.3(c)). The result of such activation might be the appearance in the cells of enzymes which could cause a shift in the metabolic pattern, such as to promote the formation of components required for the processes of growth and moulting.

c. The Corpus Allatum

The corpus allatum is a compact tissue composed of dense clusters of glandular cells; in the active condition, the cells are relatively rich in cytoplasm, and contain aggregations of glycoprotein granules. During the resting phase there is a decrease in cytoplasmic volume (as illustrated in Fig. 14.4) and the cell membranes tend to become deeply folded.

The corpus allatum is active in the control of reproduction (see below) and one of the most fruitful sources of the hormone that it secretes has proved to be the abdomens of adult, male giant silkworm moths of the genus *Hyalophora*. Extraction of abdomens with lipid solvents yields an oily, orange liquid, which shows high activity in commonly employed assay systems for juvenile hormone. If, for example, it is applied to punctures in the pupal cuticle of the mealworm beetle, it induces the formation of a larval type cuticle, instead of the adult type, at the next moult. The development of convenient assay techniques like this has facilitated the separation and purification of active materials from a variety of sources, including not only insects but vertebrate tissues, as well as bacteria and plant material. Activity was found to be associated with open-chain terpene components, and the active principle was originally identified as farnesol and its aldehyde derivative, farnesal; recent work, however, has established the identity

of the active principle in *Hyalophora* extracts as the related compound, methyl *trans, trans, cis*-10-epoxy-7-ethyl-3,11,dimethyl-2,6-tridecadienoate, the structure of which is illustrated in Fig. 14.4(c) (Röller *et al.*, 1967). In its ability to ensure the retention of juvenile characters in a variety of assay systems this compound is more than a thousand times as effective as farnesol, and there seems no reason to doubt that it constitutes the true juvenile hormone.

Fig. 14.4. The corpus allatum. a, histological appearance of cells of the inactive corpus allatum of *Leucophaea madera*; the spindle-shaped cells are closely packed and have little cytoplasm; b, histological appearance of cells in the active corpus allatum, showing a substantial increase in cytoplasmic components (drawn from photomicrographs of Engelmann, 1957/8); c, the chemical structure of the juvenile hormone (Röller *et al.*, 1967).

The effect of juvenile hormone in the developing insect is to ensure the production of larval, or juvenile, characters. This has been convincingly demonstrated by experiments in which the source of the hormone in young larvae of the silkworm is removed by operative procedures. In the absence of the corpus allatum, and hence of juvenile hormone, the larvae moult to form precocious pupae of minute dimensions, weighing no more than 2.5 mg, as compared with the normal weight of 1.25 g. The precise mode of action of the hormone has not yet been determined, but it may involve a direct effect on the gene system, such as to activate those elements of the total system that are associated with the formation of larval characters, at the same time suppressing the activity of those that are responsible for the formation of pupal and adult features. The effect seems to be graded, in the sense that the final outcome depends on the precise titre of juvenile hormone during the active period, as illustrated in Fig. 14.5. If the titre is high throughout the active phase, the immature characteristics are fully realized. If the titre is lower, or if high titres

are not present until comparatively late in the cycle of activity, then a condition intermediate between the immature and the adult form may be produced; while if the titre of juvenile hormone remains low throughout the active phase of growth, then larval characters are completely suppressed, and those parts of the gene system that are responsible for the formation of adult characters are allowed full expression.

One very important aspect of the activity of the juvenile hormone is the maintenance of the structural integrity of the thoracic gland. This gland may, in fact, be considered as an immature character, since it is the moulting hormone which it produces that ensures the continuation of growth and moulting, an essentially juvenile process; without a supply of moulting hormone, this process would inevitably come to a stop. In the presence of juvenile hormone, the thoracic gland will, at the end of its cycle of activity, enter upon a resting phase in preparation for the next cycle. But if the thoracic gland goes through a cycle of activity in the absence of juvenile hormone, then the gland breaks down completely. Within 24 hr of the final moult in *Rhodnius,* for instance, the nuclei of the thoracic gland can be seen to be undergoing chromatolysis, and after 48 hr they have disappeared completely; the disintegrating fabric of the gland is concurrently invaded by phagocytic haemocytes, which help to dispose of the cellular debris (see Fig. 14.3(a)). Here it is not just a question of allowing the expression of one potentiality, while suppressing another, but rather of a complete shut-down of the gene system. With the disappearance of the thoracic gland, and the consequent cessation of growth and moulting, the corpus allatum is free to resume activity, and it now becomes effective in the control of reproduction rather than of development, as will be described below.

The mechanism by which the secretion of juvenile hormone by the corpus allatum is controlled has not yet been fully elucidated. Its activity appears to be low just before the larval moult, rising to a peak just after the moult; it becomes completely inactive after the pupal moult, and remains so until two-thirds of the way through the pupal period in the silkworm. The indications are that a restraining influence on the corpus allatum is exerted by centres in the brain, but a great deal more work remains to be done on this aspect of the problem.

On the basis of the results reviewed in this chapter, it would seem that the growth and development of insects could be interpreted, as a first approximation, in terms of an interaction between the system's genetic basis and two hormones, the moulting hormone and the juvenile hormone. The secretion of moulting hormone causes an activation of synthetic systems leading to the formation of new substance, as the indispensable basis for the process of growth; while the intensity and timing of secretions of juvenile hormone govern the type of substance that is formed, whether larval or adult (see Fig. 14.5). There is evidence, however, that other hormones may be involved in the control of certain aspects of the process. Hardening and darkening of the newly formed

Fig. 14.5. Diagram illustrating the hormonal control of metamorphosis in epidermal cells of a hemimetabolous insect acted upon by moulting hormone (open rectangles) and juvenile hormone (hatched rectangles) in different proportions (redrawn from Wigglesworth, 1965).

cuticle, for instance, appear to be controlled independently of other processes involved in ecdysis. The active factor is released from neurosecretory cells of the brain, apparently in response to nervous stimuli from the thorax.

2. Neuroendocrine Control of Reproduction

The resumption of activity by the corpus allatum which follows the completion of development, and to which reference has already been made, appears to be an essential feature in the control of reproduction. In allatectomized females, oöcyte development only proceeds to the point at which yolk would normally be deposited, after which a process of resorption supervenes. The secretions of the corpus allatum appear also to play an important part in the male, with particular reference to the activity of accessory glands and the formation of spermatophores.

There are indications that the material secreted by the corpus allatum may serve a more substantive function than that of chemical messenger. This is suggested, for instance, by the close correlation which has been established between ovarian activity and corpus allatum volume, as illustrated in Fig.

14.6(a), which indicates that the gland might actually be furnishing raw material for the formation of yolk; and also by the fact that removal of ovaries has been shown to cause hypertrophy of the corpus allatum in several species, as if material which would normally be used for oöcyte development has under these circumstances had to be retained by the endocrine gland. It would be in accord, too, with the observation that male moths serve as a richer source of juvenile hormone than females, which contain only a fraction of the male activity; and

Fig. 14.6. Neuroendocrine control of reproduction in female insects. (a) The relation between ovarian activity and the volume of the corpus allatum in *Leptinotarsa* (de Wilde, 1964). (b) Diagram illustrating the factors involved in the control of reproduction in *Leucophaea*. Mating impulses are transmitted via the ventral nerve cord to the proto-cerebrum, resulting in a release of corpus allatum activity and subsequent induction of follicular activity. During pregnancy, the corpora allata are inhibited, presumably by nervous and humoral mechanisms resulting from the presence of an egg case in the brood sac (de Wilde, 1964 after Engelmann).

with the fact that implantation of ovaries into male pupae results in a marked decrease in the content of juvenile hormone, suggesting that the developing ovaries in some sense "consume" the active principle, whatever the precise significance of the utilization may be.

In many insects, the development of ovaries is closely linked with the nutritional state, as one might expect in view of the demands that oöcyte development would make on food reserves; in the absence of nourishment, oöcyte development may, in fact, be suppressed completely. In this effect, too, the corpus allatum has been firmly implicated, since the activity of the gland itself can be shown to be governed by nutritional state; and if active corpora allata are implanted into starving insects, ovary development commences.

While the corpus allatum appears to exert a major influence on oöcyte development, it is by no means the only important factor. Allatectomized females of *Calliphora* will occasionally produce fully developed eggs, but they fail to do so if the neurosecretory cells of the brain are removed. Reimplantation of corpora allata fails to restore ovarian activity in such insects, but implantation of neurosecretory cells, or of corpora cardiaca, leads to development of the ovaries. It would seem that the normal function of the corpus allatum is dependent on connection with the neurosecretory cells, and that in the over-all regulation of reproduction, there is a complex interplay of nervous and humoral factors, involving protocerebrum and lower nerve centres as well as corpus allatum, corpora cardiaca and the ovaries themselves. By virtue of such central nervous participation, ovarian activity will come under the influence of a variety of environmental factors associated with reproduction, and specifically related to the biology of the insect concerned. For this reason, it is difficult to generalize the situation, and it will be necessary to consider a concrete example in order to illustrate the interaction of factors involved in the control of reproduction.

One of the most thoroughly investigated species is a viviparous cockroach, *Leucophaea madera,* whose reproduction shows a particularly striking interaction of nervous and humoral influences (see Fig. 14.6(b)). In this species, the activity of the corpus allatum appears to come under the restraining influence of higher nerve centres. The act of mating releases the corpus allatum from nervous inhibition, an effect which is mediated through the ventral nerve cord, and the development of oöcytes proceeds to completion under the influence of its secretions. The disinhibited corpus allatum enters upon a phase of cyclical activity correlated with successive waves of oögenesis, suggesting the existence of some sort of feedback from the ovaries. Ovarian development is arrested during the period of embryogenesis, as the result of what appears to be activity in a dual control system; the presence of eggs in the brood sac seems to cause a nervous input to the ventral cord, to produce an inhibitory effect on the corpus allatum via the nervi corpora cardiaca; additionally, there appears to be a

direct endocrine effect, because the disinhibition which can be produced by removing the egg case from the brood sac can be reversed by implanting the egg case in the body cavity; and severing of the ventral nerve cord is not as effective in activating the corpus allatum as is egg case removal. Following completion of embryonic development, the act of parturition provides an input to the ventral nerve cord which has the same effect as mating, and causes the corpus allatum to be released from inhibition, thus re-starting the cycle of reproductive activity.

3. Other Neuroendocrine Control Mechanisms

The regulation of growth and reproduction provides particularly striking examples of the working of neuroendocrine control mechanisms, but there can be little doubt that many other physiological activities are regulated, though perhaps less spectacularly, by similar means. It seems, indeed, that reproductive hormones may have quite general effects on the metabolism of adult insects, causing, for instance, an increase in the rate of oxygen consumption which has been shown to be independent of ovarian development. The question therefore has been raised, whether the control of egg production may not be simply one facet of a general effect on respiratory and synthetic metabolism. Certainly, the neurosecretory cells have been firmly implicated in the control of protein secretion by cells of the midgut in certain flies, and similar effects have been described for other kinds of insect. Their secretions appear also to exercise an important general influence on the protein metabolism of the locust, causing an increase in the level of blood protein by activating the synthetic machinery of the fat body. If these blood proteins fail to be taken up by the ovaries, there is a progressive rise in blood protein level. To what extent these, and similar, effects reflect a direct action of the hormones, as opposed to a general homeostatic adjustment between different components of the total somatic system, is uncertain at this stage, but there can be little doubt that they will prove a fruitful field for experimental investigation by modern biochemical techniques, and that the situation is capable of elucidation on this basis.

The problem of neuroendocrine control mechanisms has assumed a new dimension with the recent discovery, by Finlayson and Osborne, of the wide

Fig. 14.7. Peripheral neurosecretory cells of the stick insect. (a) Diagram of the innervation of one side of an abdominal segment. Neurones on the course of nerves are numbered from 1 to 10; fb, fat body; fbn, fat body neurone; mn, median nerve; na_1, na_2, na_{2a}, nl, nl_2, np_2, np, npa, nerves; nh, neurohaemal tissue; sp, spiracle; tns, transverse nerve swelling (Finlayson and Osborne, 1968). (b) Schematized diagram of a transverse section through a part of the link nerve (na_1 of (a)) in the stick insect. A group of nerve fibres, Nf, some of which contain granules, is contained by wrappings of the Schwann cell (Sc). A large nerve process (Np) is packed with granules and has no Schwann cell sheath. This "naked" process, whose membrane is markedly convoluted, is separated from the haemolymph (H) by only a thin coating of basement membrane material (Bm). N, Schwann cell nucleus. (Drawn from electron micrograph of Finlayson and Osborne, 1968.)

(a)

(b)

distribution of peripheral neurosecretory elements. At least 10 multi-terminal neurones have been identified on each side of each abdominal segment of the stick insect, as illustrated in Fig. 14.7(a), associated with the major nerves and tracheae. The neurone dendrites contain accumulations of typical neuro- secretory granules, and they are often separated from the haemocoele by no more than a thin coating of basement membrane material (Fig. 14.7(b)). There seems little reason to doubt that these granules are destined for release into the haemolymph, but further experimental work will be required to determine the factors which cause release, and the nature of the target of action. The picture which is suggested by these preliminary results, however, is of a diffuse pattern of neuroendocrine control, of which one demonstrated example would be the diuretic effect of hormones from neurosecretory elements associated with the abdominal nerve cord of *Rhodnius,* which has been mentioned earlier. It is possible that the diffuse nature and wide distribution of many neuroendocrine elements could usefully be seen as another reflection of the inefficient pattern of blood circulation which characterizes most insects (see Chapter 3). The corpora cardiaca could be interpreted as a condensed form of what is generally a diffuse system, situated in a region of maximum condensation of segments, and responsible for the regulation of activities whose time scale is so extended that efficient distribution of neurosecretory material would not be a primary requirement.

Aspects of Physiological Ecology

INTRODUCTION

The first three sections of this book have dealt with the physiology of insects as elucidated by experiments carried out under standardized and closely controlled conditions. What has been described, essentially, is the physiology of the individual insect in the laboratory; what could be considered to be of far greater interest and importance would be an account of the physiology of the species in its natural environment. Such an account would clearly have to be based on knowledge gained through an experimental analysis of different physiological processes under laboratory conditions, but it would go beyond this to a consideration of the way in which these processes interact in a complex and ever-changing environment; and in the final analysis, this interaction would find expression in population dynamics, with the shifting balance between birth and death determining the number of individuals present in the population at any one time. The influences which would have to be assessed in this context would include not only the physical factors of the environment, but also the interrelations between the insect and its parasites and predators, its symbionts and its competitors, whether intraspecific or interspecific. An account of population dynamics in such terms would perhaps represent a goal towards which all investigations of insect biology might be said to tend, but it is one which is quite unattainable on the basis of present knowledge. Even with the species that have been most thoroughly studied, both from the physiological and the ecological point of view, the complexities of the situation militate against anything more than a superficial evaluation of some of the more important factors. Nor could the concept of the "typical" insect be extended to a treatment of physiological ecology, because what is of importance here is the detail of the interaction between the insect and its environment, and this would depend so fundamentally on the particular species involved. When one considers, for instance, the diverse feeding habits of insects, ranging from the various general categories of omnivorous, herbivorous and carnivorous, through the more restricted types of sap-sucking, blood-sucking or nectar-feeding, to the highly specialized modes of feeding seen in the wax moth or the clothes moth, it is clear that the relations of insects to this particular aspect of the environment would be completely beyond meaningful generalization. The same would apply to most other features of the environment, particularly the biotic ones, but it seems possible to single out two which, because of their very direct effect on the

physiology of insects, might be capable of treatment in general terms; the one, humidity, the other, temperature. Both are so closely linked to the basis of physiological function, that insects could be expected to be affected by them in a generally similar way, and these features have accordingly been isolated for discussion from the complex of factors which in their totality comprise the environment.

If the balance between the rate of birth and death of insects is affected by humidity and temperature, or more generally, by climate, then one would expect to see the seasonal and long-term changes which characterize terrestrial environments reflected in corresponding fluctuations in the population density of insects. That striking fluctuations do occur in the density of many species of insect has long been known, though data on this aspect of insect ecology have usually to be accepted with caution. The existence of serious sampling errors is recognized by all workers in this field, but it is not often that attempts have been made to evaluate their magnitude. However, even if the precision of population estimates is usually questionable, there can be little doubt that the kind of short-term and long-term changes that are illustrated in Fig. 15.1(a) do provide an indication, however imperfect, of the real changes in population density that underlie them. Such changes can often be plausibly, and sometimes even convincingly, related to corresponding changes in temeperature or humidity. It can be seen from the inset of Fig. 15.1(a), for instance, that the apparent density of tsetse flies tends to be low at the height of the hot-dry season, and significant correlations can be obtained between apparent density and temperature or humidity (or saturation deficit, which is effectively a combination of the two, see Chapter 16). Similar correlations between physical factors, like minimum temperature and rainfall, and the number of insects caught in light traps have been obtained in temperate climates, and Fig. 15.1(b) illustrates one of the more sophisticated examples of this approach to the problem of population dynamics. Here the relation between the population density of thrips and various environmental factors has been analysed by the statistical technique of multiple regression, and a close correspondence has been established between the observed population density, and the density which can be calculated on the basis of the regression equation, by substituting for recorded values of the physical factors. This demonstrates that, for the situation described, the density fluctuations can be almost completely accounted for by the climatic fluctuations. In the mind of the sceptic, however, the question inevitably arises whether the relation between the variables is a causal one; to return to the earlier example, one may ask whether the population density of tsetse flies is low *because* the saturation deficits are high in the hot-dry season, or simply *when* the saturation deficits are high. It could well be that both saturation deficit and density are correlated with some third factor, or complex of factors, and that it is with this that a causal relationship subsists. It is

Fig. 15.1. Aspects of the population dynamics of insects. (a) Fluctuations in the density of tsetse flies. The main curve shows long-term changes in the apparent density of *Glossina swynnertoni* at Shinyanga, Tanganyika, over a period of 21 years. The inset shows seasonal variations about the annual mean, with monthly catches expressed as a percentage of that mean; a marked decrease in density occurs with the onset of the hot-dry season, followed by a rapid recovery during the rains. The apparent density represents the catch of adult males per 10,000 yards of transect, and bears a relation, though not necessarily a constant relation, to population density (redrawn from Glasgow and Welch, 1962). (b) A comparison between the observed density of thrips at the spring peak (open columns) and the density calculated on the basis of a multiple regression with climatic factors as independent variables (closed columns); for further explanation see text (Bursell, 1964b from Davidson and Andrewartha).

important to bear in mind that this doubt can never be answered by regression analysis; the regression coefficient can provide no more than an indication that a particular physical factor is important in relation to the population dynamics of a particular species, no matter how closely the situation is described by the regression equation. Unless that suggestion can be independently verified, it remains no more than a suggestion.

With this qualification, the available evidence does indicate that temperature and humidity may have a substantial influence on the population density of insects, and attention must be turned to the mechanisms by which such effects could be exerted. Any environmental factor which affects the rate at which insects are born to the population, or the rate at which they die, will have an effect on the numbers present at any one time, and hence on population dynamics. Consideration must accordingly be given, in the last two chapters of this book, to the ways in which the birth-rate and the death-rate of insects may be affected by temperature and by humidity.

TEMPERATURE EFFECTS

In the introduction to this book it has been suggested that an insect can be considered essentially as a special type of metabolic system, and this point of view is of obvious relevance to the present discussion, because the reaction rates of component parts of such a system should be related to temperature in a fairly simple way. An example is given in Fig. 15.2(a), which shows the effect of temperature on the rate of a chemical reaction catalysed by an enzyme. It can be seen that, over the lower range of temperatures, the reaction rates increase by a factor of about 2 (the precise value may vary between 1 and 4 depending on the enzyme). At higher temperatures the situation is complicated by the thermolability of most enzymes; here the curve tends first to flatten out, as the increase in reaction rate is partially offset by an increase in the rate of thermal inactivation; and then to fall towards zero, as the enzyme becomes more quickly inactivated. A competition between two rate processes with opposite effects is involved here, and the temperature at which the quantity of reaction product is maximal (the optimal temperature) will depend on the duration of the assay, being lower for long than for short durations.

If an insect can be considered as a metabolic system comprising a network of metabolic pathways, each mediated by a sequence of enzymes, then the effect of temperature on any particular life process might be expected to take a form similar to that illustrated in Fig. 15.2(a). The close resemblance between the four curves of Fig. 15.2 suggests that this may not be too naïve a point of view. Fig. 15.2(b) shows the effect of temperature on the rate of oxygen consumption of an insect, which may be considered as a process mediated by a complex enzyme system rather than by a single enzyme. Figure 15.2(c) shows the effect of temperature on the rate of development of an insect, which may be considered as the end-product of an immensely complicated network of pathways. Figure 15.2(d) shows the relation between temperature and what might be called the rate of living, a process which cannot be precisely defined, but which can be estimated as the reciprocal of longevity, in the same way that the rate of development can be estimated as the reciprocal of developmental duration; it would denote, in other words, how long the insect takes to live its

Fig. 15.2. The effect of temperature on life processes. (a) The effect of temperature on the activity of a digestive protease, expressed as the amount of amino acid nitrogen released by hydrolysis of a protein substrate (redrawn from Baldwin, 1948 after Berrill). (b) The effect of temperature on the rate of oxygen consumption of unfed tsetse flies, *Glossina morsitans* (redrawn from Rajagopal and Bursell, 1966). (c) The effect of temperature on the rate of pupal development in female *Glossina morsitans,* expressed as the proportion of total development completed in 10 days (drawn from data in Phelps and Burrows, 1969). (d) The effect of temperature on the longevity reciprocal of adult fruit flies, *Drosophila melanogaster,* as a measure of the rate of senescence (data from Loeb and Northrup, 1917).

life, how quickly it senesces. The general form of all the curves mirrors the simple enzyme relation of Fig. 15.2(a) quite accurately over the range that permits of their estimation. This range is limited by a discontinuity which is generally described as death, where the metabolic system ceases to exist as such. In Fig. 15.2(b) and (c), the curves do show a tendency to flatten out before this point is reached; in (d) no second inflection of the curve would be expected, since there would here be no question of opposing, but rather of reinforcing, rate processes; the thermal inactivation of proteins would contribute to, and probably take over from, other reactions as a direct cause of senescence and death, so that the rate of dying would tend to infinity rather than to zero.

The indication from these general relations are, therefore, that temperature would have a readily definable effect on the rates of metabolic processes leading to the birth and death of insects, and hence a predictable effect on the population dynamics of insects. Naturally, one would not suggest that all processes relevant to the birth and death of insects can be seen in such simple terms; nervous or humoral control mechanisms, which could be affected by

temperature in quite a different way, would in many cases impose other patterns on the over-all relation with temperature. Such control mechanisms would, nevertheless, operate against the background of a general metabolic relationship with temperature, and what, therefore, would be of general importance would be the temperature of that metabolic system, of the insect itself, rather than of the environment; consideration must accordingly be given first to the factors which govern the temperature of insects.

a. The Temperature of Insects

In an insect which is in thermal equilibrium with its environment, and whose temperature is therefore constant or fluctuating slightly about a steady value, the net exchange of heat between the insect and the environment is zero, the gain of heat exactly balanced by the loss of heat. Losses would be occurring by long-wave radiation, by conduction and convection, and by evaporation; gains would be by solar and long-wave radiation and by metabolism. The metabolic component arises because part of the energy released during the oxidation of complex organic molecules fails to be captured in high energy phosphate linkage and appears instead as "metabolic heat" (see Fig. 1.1 of Chapter 1). The contributions made by these various processes to the heat flux at any given moment depend so much on circumstances, that it will be convenient to consider the problem in relation to a number of different situations.

With insects at rest in the absence of solar radiation, the body temperature is usually close to ambient, and for this reason neither long-wave radiation nor conduction and convection (which occur in proportion to the temperature differential) can play much part in the heat flux. Metabolic heat is the sole source of heat, and at the low rates which characterize the respiration of resting insects, it generally fails to raise the insect's body temperature by more than a fraction of a degree above ambient. The metabolic gains of heat can be effectively balanced only by evaporative cooling, and the precise point of equilibrium will therefore depend on factors which affect transpiration, being perhaps a fraction of a degree below ambient under conditions that favour evaporation, a fraction of a degree above in humid atmospheres. The differences are so small, however, that for all practical purposes, the insect may be considered to be at the temperature of the environment.

An exception to this generalization should perhaps be made where ambient temperatures are approaching the upper critical limit. There is evidence that some insects may then increase the rate of evaporative cooling by opening the spiracles (see Fig. 15.3(a)), and in this way ensure a lowering of body temperature to a few degrees below ambient. It is clear, however, that for animals as small as insects, water reserves would be inadequate to sustain long-term regulation of body temperature by evaporative cooling, and the process described should probably be seen as something of a crisis mechanism,

Fig. 15.3. The temperature of insects. (a) The body temperature of a tsetse fly *Glossina morsitans* exposed to ambient temperatures rising from 33° to 45° and then falling to 43°. Curve (i) shows the temperature during exposure in a saturated atmosphere; under these conditions, the insect's temperature differs by no more than 0.1-0.2° from ambient. Curve (ii) shows the situation when the insect is exposed in dry air, and it can be seen that up to a temperature of 35° there is little difference between the two curves, but at higher temperatures curve (ii) drops to a position about 2.0° below curve (i), indicating that evaporative cooling becomes a factor under these circumstances. Simultaneous observation of the spiracles showed that they were held closed until the temperature reached about 35° (hatched circles at bottom of graph), with "fluttering" (half-hatched circles) at higher

brought into play to stave off, for a limited period, the point at which tissue temperatures reach the critical limit.

When insects at rest are exposed to sunlight, the heat input from solar radiation becomes a dominant element in heat balance, and body temperatures may rise well above ambient. As substantial differences in temperature develop between the insect and its surroundings, convection becomes of importance in determining the equilibrium value, losses by conduction, evaporation and long-wave radiation playing relatively little part. The factors of importance in determining the equilibrium temperature will, therefore, be those that affect the rate at which heat is gained by radiation and lost by convection, and the most important of these are:

(i) size (see Fig. 15.3(b)); the larger the insect the greater the temperature excess to which it attains at a given level of radiation input. The insect's shape, and its orientation in relation to the sun's rays are also of considerable importance, while colour appears to be relatively unimportant;

(ii) the occurrence of air movements, which determine whether heat loss occurs by natural convection, relatively inefficient as a mechanism of cooling, or by forced convection, which is much more effective (see Fig. 15.3(b)). In this context, the nature of surface covering is also of considerable importance; a dense coat of hair or of scales, as in certain moths for instance, will greatly reduce the loss of heat by forced convection.

With insects in flight, a special situation arises, because of the enormous increase in metabolic rate, and hence in the rate at which metabolic heat is generated; temperatures of the thorax may increase to as much as $12°$ above ambient in flying insects. Despite the opening of spiracles and the vigorous ventilation of the tracheal system that occurs during flight, evaporation appears to play little part in dissipating the metabolic heat, most of which is lost by forced convection from thoracic surfaces. Since flight is a very intermittent activity in most insects, it seems likely that the changes in body temperature with which it is associated may be too ephemeral to affect mean tissue temperatures substantially, and they would therefore be of little importance in relation to the rates of birth and death in insect populations.

This brief review of the factors that affect the temperature of insects suggest that they have few powers of direct regulation of body temperature, which is

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temperatures giving way to sustained opening (open circles) above $40°$. Exposure of tsetse flies to temperatures in excess of $43°$ for 1-2 hr usually causes death, and the graph shows that by evaporative cooling the insect is capable of maintaining its body temperature below this critical value, under the conditions shown (schematized from Edney and Barrass, 1962). (b) The effect of wind velocity on the temperature excess of large *(Phormia)*, medium *(Syritta)*, and small *(Drosophila)* flies, exposed to a radiation of 1.5 cal/cm²/min (Bursell, 1964 from Digby). (c) The distribution of *Grylloblatta* and *Gryllus* in a gradient of temperature at high relative humidity (Bursell, 1964 after Jakovlev and Krüger, and Henson).

perhaps not surprising in view of their small size. The only source of heat which could be considered to be under the insect's direct control is metabolic heat, and because of its small size, the surface area from which heat is dissipated by convection is large in relation to the mass of respiring tissue, so that, except under the special circumstances of flight, the temperature excess which can be maintained is slight. Similarly, the only way in which insects could directly regulate losses of heat would be by increasing evaporation, and their scant water reserves would militate against the sustained use of such a mechanism. But the fact that they have minimal powers of direct regulation does not imply that they are in any sense at the complete mercy of the environment, for they possess considerable powers of indirect regulation. Even in the absence of solar radiation, most terrestrial environments are extremely heterogeneous in respect of temperature, and there may be substantial differences between closely adjacent parts. It has been mentioned that insects possess sense organs which are capable of responding to changes in temperature, and there is abundant evidence that such sense organs are put to use in selecting for habitation those parts of the general environment which are most suitable from the point of view of heat balance. Examples of the quite narrow thermal preference shown by certain species of insect are illustrated in Fig. 15.3(c); one of the species tends to aggregate in cool parts of a gradient, the other in warm parts. In addition to being able thus to take advantage of such temperature differences as may exist in the environment, many insects habitually bask in the rays of the sun, and by this means maintain a body temperature well in excess of ambient. These considerations suggest that the mean temperature of an insect's tissues may be widely different from, and probably far more favourable than, that of the general environment. It is clear, too, that to make even an approximate assessment of the mean temperature experienced by individuals of a population in a given species would pose a formidable technical problem. Yet it is on such an assessment that one would have to rely in any attempt to gauge the effect of temperature on population dynamics. Until such time as suitable methods are devised for the continuous monitoring of tissue temperatures in representative samples of a population, it will be impossible to make more than a general guess at the part that temperature may play in the regulation of insect numbers. The basis of even such a general guess would have to be a knowledge of the effects of temperature on various physiological processes, and it will therefore be useful to discuss the general way in which such effects would operate.

b. The Effect of Temperature on Birth-rate

The two main ways in which temperature influences the birth-rate of insects is through effects on the rate of reproduction and on the rate of development. Both appear to be rather delicate processes, to judge by the fact that they occur over a rather narrower range than many other physiological processes. The

extent of the range, and its position, varies considerably from species to species, but in all there is a tendency, as the temperature increases from the lowest level which will permit the processes to occur, for the rate of oviposition and speed of development to increase towards a peak near the upper extreme. The rate of oviposition usually declines well before the upper limit is reached (see Fig. 15.4(a)), falling gradually to zero. The rate of development, on the other hand, seldom has a clear optimum, but tends to level off, or decline slightly, just before the upper extreme is reached (see Fig. 15.2(c)).

In their influence on birth-rate, these two effects will reinforce each other over lower parts of the range in the sense that, if an increase in temperature from $15°$ to $25°$ leads to an increase in the rate of oviposition from 0.2 to 1.0 eggs per day, and to a decrease in the duration of development from 20 days to 4 days (associated with a corresponding increase in the speed of development), then the rate at which adults are added to the population is effectively increased from $0.2/20 = 0.01$ to $1.0/4 = 0.25$ individuals per day. In other words, a five-fold increase in both rates leads to a 25-fold increase in the effective birth-rate. It is, of course, not legitimate to use such a simple approach, except to give a general indication of the magnitude of effect which may be expected, since it ignores the possible effect of temperature on associated factors, such as the length of the reproductive period or the mortality of developmental stages. The example simply serves to indicate that the rate at which adults will be recruited to a population of insects will be very markedly affected by temperature, and the relation with temperature would be of the general form described.

c. The Effect of Temperature on Death-rate

In the context of death it is useful to consider two types of effect; those which operate at extremes of the range, to kill the insect directly by processes which are as yet imperfectly understood; and those which operate within the viable range of temperatures, either directly, as in the phenomenon of senescence, or indirectly, in ways which will be described below.

(i) Critical Limits of Temperature. During the last few decades, advantage has been taken of the development of suitable statistical and experimental techniques to make a careful assessment of the critical limits of temperature in various species of insect. Much of the earlier work in this field suffered from a failure to evaluate in detail the relation with time, a relation which must be considered of paramount importance. At high temperatures a process, possibly of protein denaturation, is involved, which has an extremely high temperature coefficient, with correspondingly marked changes in time course over a narrow range of temperatures near the critical limit; while at low temperatures, where the formation of ice crystals may be a cause of death, the probability of occurrence of favourable molecular configurations will be a close function of

(a)

(b)

(c)

time. To get meaningful results, it is therefore necessary to study the thermal death point with minute reference to the duration of exposure. This is illustrated in Fig. 15.4(b), which shows the relation between temperature and the duration of survival at upper and lower limits for two different species of insect. Had an exposure of, say, 10 min been adopted as standard, the data show that the critical temperature for the mosquito would have been assessed as 41°, while for an exposure of 1 min, the value would be 2° higher, and similarly for *Cephus* at the lower range. In view of this relation, the best basis for evaluation would probably be to expose the insects to a given temperature near the critical limit for a range of durations, which would give a series of "dosage" levels at each of which the percentage mortality is assessed. The results can then be conveniently analysed by statistical techniques which have been developed in connection with toxicity tests, to give an accurate estimate of the time required to ensure the death of 50% of the individuals. An example of results obtained in this way is shown in Fig. 15.4(c).

The phenomenon of acclimation is one which must be taken into account in any study of critical temperatures. It has been shown for several species of insect that the critical temperature may vary according to the thermal history of the population from which the sample is taken. This is illustrated in Fig. 15.4(c), which shows that insects reared at 23° show 50% mortality (the corresponding probit value is 5.0) when the exposure time to a test temperature of 43° is 105 min, while half the individuals reared at 29° can survive for 154 min under these conditions; it shows also that a brief conditioning exposure to a temperature of 36° on the day before testing is even more effective in raising the resistance than is prolonged maintenance at 29°.

The carefully standardized work which has been done during recent years has lent precision to earlier indications that, for relatively short exposures of up to an hour or so, the upper critical temperature for insects in general is in the region of 40-45°, with tropical species usually more resistant than temperate species, and with certain specialized forms capable of withstanding extraordinarily high temperatures, in excess of 50°.

Over the lower range, where result may also be affected by the phenomenon of acclimation, the situation is complicated by the existence of different types of insect, differing in the nature of their response to low temperature. Some are

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Fig. 15.4. Effects of temperature on the rates of birth and death. (a) The rate of oviposition of *Toxoptera graminum* and *Brucus obtectus* as a function of temperature (Bursell, 1964 from Wadley and from Menusan). (b) The relation between duration of exposure and the temperature at which 50% of individuals die; upper curve, *Anopheles* exposed near the upper critical limit (redrawn from Bursell, 1964 after Platt, Collins and Wilberspoon); lower curve, *Cephus* exposed near the lower critical limit (redrawn from Bursell, 1964 after Salt). (c) Mortality curves for *Dahlbominus fuscipennis* exposed to a temperature of 43°C at high relative humidity; (a), reared at 23°; (b), reared at 29°; (c), reared at 23° and exposed for 2 hr to 36° on the day before testing (Bursell, 1964 after Baldwin).

killed by exposures of about an hour to temperatures well in excess of zero, the cause of death being as yet uncertain. Others can withstand sub-zero temperatures so long as the formation of ice crystals in the tissue fluids is prevented; disruption of the submicroscopic architecture of the cells, which would occur under these circumstances, would probably constitute the immediate cause of death; for such species, the lower critical limit would be set by the point to which their tissue fluids would be capable of super-cooling. The third category of insect is capable of surviving the formation of ice crystals in their tissues; this ability appears to be associated with the presence of high concentrations of glycerol, which may accumulate to a level of 25% of the total wet weight of the insect. The mechanism by which glycerol exerts its protective effect has not been unequivocally established, but there are indications that the lethal factor may be the marked increase in the concentration of salts which occurs as ice crystals form, rather than mechanical damage, and that glycerol serves to "buffer" the tissue fluids against this concentration effect (e.g. Lovelock, 1953).

By virtue of the existence of these three general types of cold-hardiness, the range of lower critical temperatures for the group as a whole is much higher than for the upper critical limits, extending from well below $-35°$ in several species of hibernating insect from temperate and arctic regions, to temperatures above $+5°$ for tropical species like the tsetse fly.

(ii) Effects Within the Viable Range. The criterion used for determination of upper and lower critical limits of temperature is usually death within a limited period of time following exposure. It is likely, however, that temperatures which are not critical in this sense may yet materially affect the subsequent expectation of life. If this is so, then the distinction between effects at the limits of the viable range and within the viable range may be to some extent arbitrary, reflecting no more than a habit of considering the problem in terms of mortality rather than of survival. It is certainly clear from the results shown in Fig. 15.2(d), that temperatures within the viable range may have a profound effect on the longevity of insects, indicating that what one sees at the extremes of the range may be, in part, extensions of effects which are exerted in smaller measure at other points of the range. It may not be too uncharitable to suggest that the tendency to concentrate on upper and lower critical limits may be an expression more of the technical ease of the experimental procedure than of the intrinsic importance of such limiting effects. It is much easier to determine how many insects have died after a short exposure to a test temperature than it is to trace the development of sub-lethal influences resulting from prolonged exposure to sub-lethal temperatures.

Apart from the direct effect of high temperature in curtailing the expectation of life, there may be indirect effects which could be of great importance under

natural, as opposed to laboratory, conditions; these would be associated with the increase in metabolic rate which occurs when temperatures are raised, as illustrated in Fig. 15.1(b). The rate at which oxygen is consumed reflects the rate at which food reserves are expended, and under conditions where the replenishment of reserves is intermittent, and the availability of food uncertain, a marked effect on life expectancy would be likely. To take a concrete example of what may be a general condition, the average fat reserves of a tsetse fly in the field would sustain life for about 5 days at a temperature of $20°$, but for only 2 days at a temperature of $30°$. In the cold season, the time available to the insect for locating a host animal from which food reserves could be replenished would therefore be twice as long as in the hot season and, other things being equal, so would its chance of finding a suitable host before dying of starvation, and thus of extending its life span by another hunger cycle. Indirectly, therefore, the expectation of life might be a close function of temperature.

d. Conclusion

The results presented in this chapter have demonstrated that through its direct influence on various metabolic processes, and on the nervous and humoral control systems which regulate the processes of reproduction, temperature may exert a profound effect on the rate at which insects are born and die to their populations. It is possible to gain some insight into the integrated effect of all these influences under laboratory conditions by the construction of life tables for a laboratory colony, from which age-specific fecundity rates can be calculated. From such data, the innate capacity for reproduction (denoted by the symbol r_m) can be estimated over a range of temperatures; this measures the rate at which females of reproductive age are added to a population of stable age distribution. Thus, instead of getting estimates separately of fecundity (Fig. 15.4(a)), speed of development (Fig. 15.2(c)) and length of life (Fig. 15.2(d)), a measure is obtained which represents an integration of these, and other, relevant processes in terms of reproductive potential. In the few species where this has been done, the relation with temperature is roughly as one would expect from a consideration of the component processes, with an inflected rise in the value of r_m from low levels at low temperatures to a peak, followed by a steep fall towards upper limits of the range. Unfortunately, this elegant approach to the problem suffers from a number of severe limitations. In the first place, it can only be confidently employed when the insect concerned is fully amenable to laboratory maintenance, and this is not the case with most species. Secondly, the evaluation is made under conditions which differ widely from those that prevail in the natural environment, particularly in such respects as the availability of food and water. Where these constitute limiting factors to survival, the results of laboratory investigations cannot be applied with any confidence to the natural

state; for they are obtained with populations which are allowed to live their full life span, while, under natural conditions, causes of death other than old age are likely to exert a dominating influence on life expectancy.

Even if an accurate assessment cannot be made of the precise effect of temperature on reproductive potential, there can be little doubt that the general relation between them will be of the type described. It is known that the temperature of terrestrial environments is subject to substantial diurnal and seasonal variation, and may swing in the course of weeks or months between upper and lower limits of the viable range. Through their behavioural reactions to temperature, and by virtue of the heterogeneity of terrestrial environments, insects would be able to inhabit those parts of the environment that are most suitable to their life processes; and where they cannot escape exposure to sub-optimal conditions, they can to some extent buffer themselves against the effects of such exposure by their powers of acclimation. But despite these abilities, there can be no doubt that the seasonal changes in environmental temperature will be reflected, though to a degree which cannot at present be precisely gauged, in corresponding changes in the temperature of the insects themselves, and hence that corresponding effects on their population dynamics are likely to result. A proper insight into the quantitative details of such effects must await substantial advances in the investigation of physiological as well as ecological aspects of the problem.

HUMIDITY EFFECTS

The relation between environmental humidity and the population dynamics of insects differs from that which has been described between temperature and population dynamics, because with humidity there is no question of a viable range, or of critical extremes. Humidity has no direct effect on the metabolic system in the way that temperature has, and extremes of humidity do not, in themselves, kill an insect. The effect that humidity has on insects is mainly an indirect one, through water content; if that is reduced below certain critical limits by exposure to desiccating conditions, or, conceivably, if it is raised above a certain limit, then the insect dies; and short of these limits a variety of sub-lethal influences may be exerted on processes capable of affecting the rates of birth and death. What is of importance, therefore, is to see water balance as a cumulative function, and in this respect unlike heat balance, which is essentially an instantaneous function. The differential between water losses and water gains must be regarded as adding up over a period of time, with a gradual depletion of reserves, perhaps, until the critical limit is reached. And in so far as this is the case, the important feature of the environment is not the amount of water vapour present in the atmosphere, its percentage saturation as expressed by relative humidity, but rather the amount of water vapour which is lacking from the atmosphere; for it is this that will determine its power to promote the evaporative loss of water. Unfortunately, the evaporating power of an environment is not a characteristic that can be easily measured. In the first place, it is closely related to temperature, since the saturated vapour pressure of air increases greatly with an increase in temperature, and so, therefore, does the saturation deficit, as illustrated in Fig. 16.1. The saturation deficit is a measure of the difference between the amount of water vapour present in the atmosphere and the amount which would be required to saturate the atmosphere at any particular temperature, and to this extent it provides a useful indication of evaporating power. At any given saturation deficit, however, the evaporating power may be substantially influenced by air movements, which will tend to steepen gradients of water vapour between the evaporating surface and the general atmosphere. This effect will be especially important when rates of

Fig. 16.1. The relation between vapour pressure and temperature at full and at half saturation. The saturation deficit at any temperature is given by the vertical distance between the two curves. (Bursell, 1964.)

evaporation are relatively high ("vapour limited" as opposed to "membrane limited" systems in the terminology of Beament, 1961). The general level of evaporation is of importance also in another respect, because it will govern the temperature of the transpiring surface, which at high levels of evaporation may be several degrees below ambient. It is clear that the evaporating power of a given environment, as it governs the rate of water loss from a given insect, cannot easily be assessed by measurements of the relevant physical features of the environment. Perhaps the best approach to the problem is the more direct one of measuring the rate of evaporation from a simple physical model, designed to simulate the organism under investigation in respect of surface area and general level of water loss. This method has been used with considerable success in an investigation of the spruce bud worm in Canada (Wellington, 1949), but most workers have been content to arrive at an approximation to the evaporating power by basing their results on measurements of saturation deficit.

In attempting to define the ways in which the evaporating power of the environment may affect the survival of insects, what needs to be considered first of all is, what exactly is the critical level of water content, or better, what is the extent of the water reserve, defined as the difference between the water content

of the critically depleted and the fully hydrated insect, with allowance made for the production of metabolic water (see below). Secondly one would require to know what are the factors that influence the rate at which insects lose and gain water. It is in the relation between the extent of the reserve and the rate of its depletion that the effect of humidity on the rate of death must primarily be sought. In so far as humidity may affect the rate at which eggs are laid, and at which development proceeds, it will also have an effect on the rate of birth, but here the relation cannot usually be interpreted simply on the basis of a depletion of water reserves, and the problem must be tackled empirically.

1. The Water Reserves of Insects

The water reserve of an insect may be defined quite simply as the amount of water that the insect can afford to lose before the critical level of water content is reached. It should, on the face of it, be a simple matter to determine this quantity experimentally, but unfortunately the problem is complicated by a technical difficulty, so that, in fact, little accurate information is available in the literature on this point. Since insects are able to lose a certain proportion of the water that they contain, the actual amount of water that can be lost will obviously depend on their size. This applies both within species and between closely related species, as illustrated in Fig. 16.2. In order to determine the quantity of water reserve, allowance must therefore be made for differences in size between individuals in a sample, or for differences in mean size between samples. This is usually done by expressing water content as a proportion, or percentage, of some function of size, usually total fresh weight. Unfortunately, this procedure introduces a number of complications. It has been shown, for instance, that fat constitutes one of the most important food reserves in many insects; it would be possible to maintain an insect of this kind under conditions where losses of water were exactly counterbalanced by gains; the amount of water present in the insect would be constant, yet its water content, expressed as a percentage of fresh weight, would show a steady increase as the amount of fat decreases. Such anomalies could be partly circumvented by expressing water content as a percentage of the non-fatty dry weight, as has been done in Fig. 16.2, but this does little to improve the situation if substantial quantities of non-fatty reserves are also expended. The best way to overcome these difficulties would be to use some constant linear dimension as a measure of size, but this has seldom been done, and available data cannot, therefore, be used as a basis for an accurate assessment of the quantity of water reserve in insects generally. All that can be said is, that the water content of fully hydrated adult insects is usually in the region of 75% of the non-fatty dry weight, while critical levels of water content are in the region of 60-64%. This means that losses can be sustained of just about half of the water present in the fully hydrated insect (Fig. 16.2). The

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Fig. 16.2. The relation between size and the quantity of water reserve in different species of tsetse fly. Curve (i) shows the water content of flies which have newly emerged from pupae maintained at high relative humidity; curve (ii) shows the water content of flies that have been desiccated to the point where they are no longer capable of righting themselves. The difference between the two curves shows the extent of the water reserve, which is about 45% of the water originally present. ⊞, *Glossina austeni*; ▲, *G. palpalis*; ⬛,*G. pallidipes*; ◑, *G. brevipalpis*;◆, *G. fuscipleuris.* (Simplified from Bursell, 1959.)

survival of the insect will depend on its ability to maintain a balance between losses and gains of water such that the reserve of water never becomes fully depleted.

2. The Water Balance of Insects

Insects can lose water by transpiration and by excretion, and they can gain water by ingestion and by the production of metabolic water. Some information relevant to a consideration of water losses and water gains by these processes has been given in earlier sections of this book (see Chapters 1, 5 and 7); in this chapter an attempt will be made to bring together the information on this subject, so as to provide a general picture of water balance in terms of the interaction of component processes.

a. Losses of Water

It is necessary to reiterate at the outset the difficulties that beset insects, in view of the fact that they are small animals inhabiting a terrestrial environment;

animals whose surface area, through which water vapour can be lost to the general environment, is large in relation to the water reserves which must sustain that loss. A primary requirement for animals faced with this difficulty would be an effective water-proofing of the surface, which would serve to reduce the loss by transpiration. A limit would be set to the extent to which an animal could thus insulate itself from the desiccating influence of the environment, by the need to provide for respiratory exchange, and for the excretion of waste products. These exchanges would have to be superimposed upon the general background of impermeability to water, and one would expect to find, associated with these points of exchange, the development of stringent control mechanisms to minimize the losses of water associated with them.

The permeability of the cuticle of insects to water is, in fact, extremely low, and water-proofing has been shown to be associated with the presence of lipids in the epicuticle (see Chapters 1 and 13). The rate of transpiration is, therefore, enormously increased if the epicuticular lipids are disrupted by exposure to lipid solvents or detergents, or to the action of abrasive dusts. It appears that the main barrier to the diffusion of water is provided by an orientated monolayer of lipid molecules, situated at the surface of the cuticulin layer, supposedly with their polar groups associated with the hydrophil surface, and their non-polar hydrocarbon chains extended outwards at an angle to that surface. It is this involvement of lipids as the basis of water-proofing that imposes a characteristic relation between temperature and permeability (see Fig. 16.3); rates of water loss show a sharp increase when the temperature of the transpiring surface reaches what appears to correspond to the melting point of cuticular lipids. This transition is presumably associated with an increase in the mobility of the lipid molecules, leading to changes in molecular spacing. It is unfortunate that it has not yet proved possible to relate this interpretation to the actual composition of the cuticular lipids. Cuticular extracts have always been found to comprise a complex mixture of lipids, only some of which conform to the requirements of the model for molecules with one end polar and the other non-polar. It is possible that it is only that fraction of the total which answers to this requirement that contributes to the orientated monolayer, but if that is so it is difficult to understand why there should be such a close correspondence between the transition temperature and the melting point of the total mixture.

The breakdown of water-proofing at the transition temperature is probably of limited biological significance, since in most insects it occurs at temperatures to which they would not normally be subjected, and in many it is above the upper critical limit. What is of interest from the point of view of general, as well as of molecular, biology are the enormous differences in permeability which characterize different species, as shown in Table 16.1. Usually the lowest values are found among insect eggs, where water reserves are extremely small in relation to surface area, or among pupae, where prolonged exposure to low humidities

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Fig. 16.3. The effect of temperature on the permeability of insect cuticles to water. The rate of transpiration per unit of saturation deficit remains relatively constant as temperature is raised until the "transition temperature", which differs from species to species, is reached. At this point there is a sharp and progressive increase in permeability as the temperature increases. (Beament, 1959.)

may occur under circumstances where a replenishment of water reserves is precluded. Active stages, whether larval or adult, tend to be comparatively poorly water-proofed. Within a given developmental stage, it is often possible to establish a correlation with habitat; those insects that inhabit arid environments, like the locust and the tsetse fly, being generally better water-proofed. Thus it would seem that differences between species and stages are in a broad sense adaptive, and what would be of interest would be to determine the nature of the adaptation at the molecular level, to determine what feature it is of the interaction between the lipids and their cuticular substrate that makes a difference of as much as three orders of magnitude in the cuticular permeability.

Because of the large surface area which characterizes insects generally, the losses of water sustained by cuticular transpiration constitute a substantial fraction of total loss, despite the efficient water-proofing of the surface. In a tsetse fly at rest in dry air, for instance, approximately 65% of the total water loss occurs by transpiration through the cuticle; the relatively low level of loss by transpiration from the tracheal system (20%) and by excretion (15%) is a reflection of the stringent control mechanisms which operate at these points, as already described (Chapters 5 and 7). During flight activity, the contribution from the tracheal system increases, and of the increased total loss only 30% can

TABLE 16.1

The permeability of insect cuticles, as determined by measuring the rate at which water is lost by cuticular transpiration in dry air at temperatures between 20° and 30°

Developmental stage	Genus	Permeability $mg.cm^{-1}.hr^{-1}.mmHg^{-1} \times 10^4$	Author
Egg	*Rhodnius*	30	1
	Lucilia	150	3
	Phyllopertha	600	5
Larva	*Tenebrio*	100	7
	Nematus	200	8
	Hepialus	1900	8
	Agriotes	6000	8
Pupa	*Glossina*	3	2
	Tenebrio	10	4
	Agriotes	228	8
Adult	*Rhodnius*	120	4
	Glossina	130	7
	Schistocerca	220	6
	Calliphora	390	7
	Periplaneta	550	7
	Bibio	760	8

1. Beament, 1949; 2. Bursell, 1958; 3. Davies, 1948; 4. Holdgate and Seal, 1956; 5. Laughlin, 1957; 6. Loveridge, 1968; 7. Mead Briggs, 1956; 8. Wigglesworth, 1945.

be attributed to cuticular transpiration; similar values have been reported for the locust. At high humidities, when spiracular and excretory control mechanisms are less stringently applied, the proportionate contribution of cuticular transpiration again decreases, this time in the context of a fall in total loss. These results relate to insects whose cuticles are relatively well water-proofed (see Table 16.1), and it is likely that in others the cuticular contribution may be even higher, but available information does not enable accurate estimates to be made.

To get some idea of the resistance of an insect to the desiccating influence of the terrestrial atmosphere, it is necessary to consider the losses sustained in relation to the extent of water reserves. In the unfed tsetse fly, at rest in dry air, the daily rate of loss amounts to about 25% of the water reserve, so that the insect should be able to survive for about four days without replenishing its water reserves, provided food reserves are adequate to sustain life for this period. Allowing for variations associated with activity, this estimate is in reasonable accord with values determined experimentally by the simple procedure of

confining the insects in a dry atmosphere without access to food, and seeing how long they remain alive. Similar experiments have been performed with a number of insect species, and they enable estimates to be made of relative resistance to desiccation. Such estimates, however, are of limited relevance to the problem under discussion, in so far as they relate to insects that have been artificially deprived of the opportunity to replenish their water reserves. Under natural conditions, the losses sustained under the desiccating influence of the environment would be capable of being partially or wholly balanced by gains of water, and it is to this aspect of the problem that attention must now be turned.

b. Gains of Water

Water reserves may be replenished either by drinking or by the ingestion of food containing a certain proportion of water, which will range from 70-90% in blood-sucking and sap-sucking insects to as little as 10-15% in species that live as pests of stored grain. In order to assess the part played by ingestion in the water balance of insects living under natural conditions, one would need to estimate the frequency and the extent of replenishment, and for most species this would pose a formidable technical problem. The only insects for which quantitative information is available are species with very specialized feeding habits. It has been shown, for instance, that the sole source of water for certain pests of stored products is their almost dry food; and that under conditions of desiccation, these insects digest only a fraction of the ingested food, which appears to be eaten in part for the sake of the water that it contains. In obligatory blood-suckers, like the tsetse fly, the only source of water is the blood of vertebrates, and it has been shown that these insects retain a greater proportion of the water of their blood meal if their tissues have been dehydrated before feeding, or if the size of the blood meal is subnormal. In the case of species such as these, where the precise composition and availability of food can be estimated, it is possible to place these findings in the context of the species' biology. But with most other insects, the situation is complicated by uncertainties concerning the quantity and nature of the food supply in the normal environment. Results are available which indicate, for instance, that locusts can maintain water balance in dry air provided that there is a plentiful supply of food and that the water content of this food is in excess of about 35% (Loveridge, 1970); but to what extent this requirement is met in the normal environment during the dry season cannot be stated on the basis of present knowledge.

Another way in which water reserves are replenished is in the process of cellular respiration, where the hydrogen of the organic molecules that serve as substrate is transported through a system of hydrogen carriers to eventual combination with oxygen, leading to the formation of metabolic water (see Chapter 1). In assessing the contribution which this source of water may make

to total water balance, it is necessary to take a number of factors into consideration. In the first place, the fact that an increase in the production of metabolic water will entail an increase in oxygen consumption, and hence in respiration, suggests the possibility that the gain in terms of metabolic water may be to some extent offset by an increase in water loss from the tracheal system. Secondly, it should be borne in mind that the gain which occurs in the course of oxidation of organic substrates cannot necessarily be counted as net gain. If food is ingested as carbohydrate, and if carbohydrate serves as the main substrate for oxidation, then it may fairly be considered that the oxidation of 1 g of carbohydrate will entail a net gain of 0.55 g of water. The oxidation of fat will produce 1.07 g of water for every gram of fat, but in this case the net gain may be very much less. The oxidation of fat produces a high yield of metabolic water because the fat contains a high proportion of hydrogen, but if, for instance, the fat reserve has been synthesized by the insect from carbohydrate or protein raw materials, then much of the hydrogen has had to be introduced into the fat molecule in the course of its synthesis, and such incorporation has been at the expense of oxidizable hydrogen, which may be considered as the equivalent of metabolic water. In this case, while the total yield of water may be 1.07 g per g of fat oxidized, the net yield would be no greater than if carbohydrate had been oxidized.

These considerations militate against any simple comparison between the three main classes of food substance. As a reserve, and neglecting the ultimate origin of the reserve, fat would appear to have the advantage of a higher calorific yield and a higher yield of metabolic water, and in a number of insects, particularly among members of the Lepidoptera and Orthoptera that indulge in prolonged migratory flights, fat constitutes a major proportion of stored foods; but further work will need to be done on all aspects of the synthesis and utilization of food reserves if the precise adaptive significance is to be unequivocally identified.

c. Summary

The results reviewed in this section have shown that, in order to survive, insects must maintain their water content within certain critical limits. It would seem that regulation of spiracular and excretory losses of water may play an important part in achieving this object, with the general level of cuticular permeability determining the range of environmental conditions over which such regulatory powers are likely to operate successfully. If the integument is very permeable, regulation may be effective over the humid end of the range, but exposure to dry conditions would probably prove inimical to long-term survival of the species. If the integument is well water-proofed, regulatory powers may sustain the species even in the driest parts of the range. In view of the limited longevity of most insects in very dry conditions, and of the fact that such

conditions are a regular feature of many terrestrial environments, it seems likely that, through an effect on water balance, environmental humidity may exert a marked effect on the death-rate of insects. Unfortunately, it is not possible to go much beyond a general statement of this sort at present, because so little is known about the quantitative aspects of water replenishment for insects living under natural conditions. Without this information it is not possible to make an estimate of the probability that members of a species would exhaust their water reserves before they have a chance to replenish them, and it is on such an estimate that an assessment of the corresponding mortality would have to be based. In the present state of knowledge, it would perhaps be more fruitful to make a more empirical approach to the problem, by determining the state of water balance in insects sampled from their natural environment. If, in a proportion of individuals, the water reserve is near to the lower critical limit, it could reasonably be inferred that death by desiccation is an important factor in the population dynamics of the species. Information of this type is available for the tsetse fly, but in this particular species the indications are that, by virtue of well-developed regulatory powers and of a low level of cuticular permeability, desiccation is not an important cause of death. It would be of interest to extend this type of investigation to species that are less well-adapted to arid environments. Until this is done, assessment of the effect of humidity on death-rate will have to rely on the indirect evidence provided by correlations between population density and environmental humidity, like the one described in the introduction to this section; or will have to be confined to a consideration of stages in the life history where the insect has no opportunity of replenishing its water reserves, as for instance with pupae or other immobile developmental stages. In these it is generally found that high saturation deficits are associated with high mortalities, with different species showing marked differences in the level of resistance to desiccation. Coupled with measurements of the conditions to which developmental stages are subjected in the natural environment, such results may provide indications whether or not environmental humidity could be expected to have a marked effect on the rate of death among immature stages of the species concerned. Unless the analysis can be extended to active stages of the life history, however, there is little hope of assessing the magnitude of the over-all effect of humidity on the population in quantitative terms.

3. Effects of Humidity on the Rate of Birth

Humidity, like temperature, may affect the rate of birth in insect populations by influencing the rate at which offspring are produced, or the rate at which they complete their development. Oviposition rates have been shown to be sensitive to humidity in a number of insects, the precise relation between humidity and egg production differing from species to species (see Fig. 16.4(a)).

Fig. 16.4. The effect of humidity on the birth-rate of insects. (a) The effect of relative humidity on the rate of oviposition in *Locusta* (curve a, left ordinate) and in *Cryptolestes* (curve b, right ordinate) (Bursell, 1964 from Hamilton and from Ashby). (b) The effect of relative humidity on the duration of embryonic development in *Lucilia* (curve a, ordinate values in hours) and of nymphal development in *Locusta* (curve b, ordinate values in days) (Bursell, 1964 from Evans and from Hamilton).

Dry conditions are generally inimical to oviposition, but while some species are adversely affected by high humidities as well, in others the rate of oviposition increases progressively to reach a maximum in near-saturated atmospheres. The mechanisms of these effects have not yet been elucidated, but it is clear that they cannot be correlated in any simple way with the water content of egg-laying females.

Humidity also has a marked effect on the rate of development in many insects. Examples are shown in Fig. 16.4(b), where the duration of nymphal development in the locust is seen to be minimal at a relative humidity of 70%, increasing steeply both at higher and lower humidities. In the blowfly, the duration of embryonic development is seen to decrease progressively as the humidity is raised from 60% to 80% relative humidity.

The variety of effects on birth-rate illustrated in Fig. 16.4 precludes a general statement of the effect of humidity on the rate at which adults are recruited to a population of insects. Each species seems to show its own peculiarities in one way or another, and each must be investigated in its own right. All that can be said is that the rate of birth will, in many if not in most species of insect, be affected by humidity.

4. Humidities of Insect Habitats

Most terrestrial environments are markedly heterogeneous in respect of evaporating power, and they can usually be regarded as a mosaic of regions of high and low relative humidity. Evaporating power would be high in regions of open and relatively bare ground, for example, with high temperatures and correspondingly high saturation deficits associated with insolation, and with evaporative losses facilitated by unimpeded movements of air. In clumps of dense vegetation, on the other hand, air movement would be reduced, shade temperatures would be low and evaporation from plant and soil surfaces could produce microhabitats of negligible evaporating power. It has already been mentioned that insects are well equipped to take advantage of the variety of conditions offered by most terrestrial environments. Well-developed reactions to humidity have been demonstrated in a large number of species, and in many, the tactic reactions which lead to their aggregation in suitable parts of the environment are reinforced by kinetic mechanisms, which tend to confine the insects to suitable microenvironments once they have been located. With species that are relatively poorly water-proofed, and which would tend to be seriously threatened by prolonged exposure to desiccating conditions, a strong positive response to moist air is generally a constant feature of behaviour, as illustrated by curve (a) in Fig. 16.5. With species that are more resistant to desiccation, there is often an interesting reversal in the reaction to humidity. Individuals that have been given the opportunity of replenishing their water reserves show a marked preference for dry air (curves (b) and (c) of Fig. 16.5), but as their water reserves suffer depletion during exposure to their preferred humidity, so the dry preference becomes progressively weaker, and eventually it is replaced by a strong preference for humid air. Such an arrangement would ensure that the insects can take advantage, while their water reserves are plentiful, of the opportunities for feeding etc. offered by the general environment, taking refuge in more restricted regions of high relative humidity as necessity arises.

Such behavioural reactions of insects to environmental humidity will clearly complicate any attempt to assess the effect of humidity on population dynamics. One would need to obtain information about the proportion of time that the individuals of a species spend in microhabitats of humidity different from that of the general atmosphere, and such information is not readily available. Considerable technical difficulties are involved in monitoring continuously the humidity of appropriate microhabitats, and the daily pattern of activity in a population of insects living in their natural environment would be extremely difficult to assess. Reliance has usually to be placed on standard meteorological measurements in the hope that, though the results may not give an accurate estimate of the level of humidity to which the insects themselves are exposed, they may yet serve to provide a reasonable general indication of that level.

Fig. 16.5. Changes with time in the sign and intensity of the humidity reaction of three species of beetle, of which *Oodes* is the least and *Harpalus serripes* the most resistant to desiccation (Bursell, 1964 from Perttunen).

It is with humidity, in other words, as it is with temperature, that we are a long way from a thorough understanding of the relation between the physical feature of the environment and the population dynamics of the insect; and the difficulty is, that while the physiological effects of humidity, or temperature, can be reasonably well assessed, we are unable to specify precisely what are the humidities and temperatures to which the insect is subjected in the natural environment. The problem is thus, basically, an ecological one, and it is to be hoped that advances will be made in the field of ecological studies capable of overcoming the difficulty.

POSTSCRIPT

A review of the main features of insect physiology, such as has been attempted here, does as much to reveal our ignorance of the subject as it does to extol our knowledge. On every page there are gaps to show how far we still are from a thorough understanding of that particular metabolic system that is an insect, and how much further we need to go before we can hope to define what it is to be an insect. This is not to say that progress has been unimpressive. In the 20 years since Sir Vincent Wigglesworth, in his Croonian lecture, celebrated the insect as an experimental system for the study of physiology, a great deal has been done to establish the basic modes of operation of its parts. During this time advances in the field of insect biochemistry have been particularly spectacular, thanks to the development of a variety of biochemical microtechniques, and much has been learnt about the metabolic peculiarities that form the basis of an insect's life. In the field of somatic physiology, too, progress has been substantial, and a particularly striking feature of recent work is the consistent demonstration of regulative control in the operation of the different organ systems. The synthesis of digestive enzymes, for instance, has been shown to be controlled in relation to food intake; the loss of water from the respiratory system appears to be minutely regulated in relation to the state of water reserves; the resorption of water and of salts from the excretory system is carefully adjusted to meet the changing requirements of osmoregulation, and so on. The mechanisms by which the controls are exerted have in many cases been identified, but little has so far been discovered about the nature of the control systems themselves, and it is in this field that one may hope for substantial progress during the coming years. Indications have already been obtained that neuroendocrine elements are involved, but much work will need to be done to identify the specific components, and to elucidate the factors that control their activity.

Perhaps the greatest challenge that faces insect physiologists at the present day is that of extending their horizon beyond the laboratory, and of moving from a study of the individual insect exposed to controlled and usually constant conditions to the population of insects living in its natural habitat. Account would have to be taken of the spatial heterogeneity of the physical and biotic environment, of the diurnal and seasonal variations of its components, and of the capacity of insects to react appropriately, both at the physiological and at

the behavioural level, to these differences in space and time. Formidable technical and conceptual difficulties would obviously have to be overcome, before such an extension of treatment could be achieved, and it is not surprising that progress to date has been negligible. It is possible, however, that an increasing awareness among insect physiologists and ecologists of the potentialities of computer technology for analysis of the complex interactions of the multitude of variables which would need to be considered may enable substantial advances to be made in this area. The practical implications of progress would be enormous in relation to the problems that face economic entomologists in the world today.

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